CYTOGENETICS AND BREEDING OF SOME ANGIOSPERMS

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TO MY PARENTS

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C H A P T E R

Chapter 1.

INTRODUCTION

1.1. General

Cytogenetics is a very important branch of Science. It describes the fundamental aspects of the science of life, it deals with the structure of the cell with special reference to the chromosomes and the genes and the relation of these to various genetic phenomenon of heredity and development. The science of cytogenetics is, therefore, of fundamental importance to mankind. It helps us not only to understand the various phenomenon concerning life but it is also of great practical importance. It is important in Agriculture, horticulture, animal breeding, in medicine and in sociology.

Cytogenetics and plant breeding of angiosperms are of great importance for humanity. Angiosperms are the source of almost innumerable products which are utilized by mankind in many ways. Almost all our food comes directly or indrectly from angiosperms. Cereals, millets, pulses, sugar, vegetables and fruits are all obtained from angiosperms. Tea, coffee, cocoa, and spices are the products of angiosperms. In addition to food, plants provide us with a great variety of fibers and timbers which are used in the manufacture of clothing and shelter. A very large number of our drugs are obtained from angiosperms.

Plant breeding of angiosperms is of special importance from the point of view of food supply. As the human population of the world is increasing rapidly day by day, adequate supply of food has become a problem. Plant breeding of angiosperms is a very important method of solving this problem.

Plant breeding aims at improving existing crop plants and in addition, its aim is at evolving new varieties and species of crop plants with a combination of desirable qualities which never existed before. One serious problem in growing food plants is their susceptibility to various diseases which reduced yield. The common method of controlling this problem is the use of various chemicals as fungicides, insecticides, pesticides etc. But these chemicals are also poisonous to man. Therefore, their use is highly dangerous. Many deaths have resulted from consuming food grains, fruits and vegetables treated with such chemicals. Even if the chemicals are used in low concentration, they may not cause death, but their continuous use is injurious to human health. Plant breeding provides a better solution of solving this problem of controlling plant disease. Plant breeding aims at evolving new crop plants that will be resistant to various disease by virtue of their special genetic constitution. If this can be achieved, there will be no need of treating the plants with chemicals that are so dangerous.

1.2. The dissertation

This dissertation is in part fulfilment of the requirement for the M. Phil degree. The dissertation presents a general account of the subject, various methods used in plant breeding including recent techniques like mutation, breeding and induction of polyploidy. The techniques of the various aspects of plant breeding are described. A review of earlier work on hybridization, mutation breeding, induction of polyploidy etc. is presented in the relevant chapters and, therefore, a separate chapter on review of literature is not included. To emphasise the results achieved with plant breeding certain specific examples have been selected instead of giving a general review. The examples selected are wheat, maize and tobacco.

Chapter 2.

2. BREEDING METHODS FOR PLANT IMPROVEMENTS

The various methods of plant improvement from genetic point of view are:

(1) Introduction
(2) Selection
(3) Hybridization
(4) Mutation breeding
(5) Induction of polyploidy

2.1. Introduction

The origins of many of the field crops grown in South and Southeast Asia and the records of their early cultivation or introduction into this area are mostly lost in antiquity. This includes each commonly cultivated crops as rice, wheat, barley, jute, sorghum, millets, pulses and sugarcane. A few of the important cultivated crops which originated into Southeast Asia at a comparative more recent date.

Early introductions were made for the most part by traders and diverse varieties and strains were imported by them. By the process of trial and error the varieties with the best ecological adaptation to the various crop producing regions gradually became known, and this use was extended in those regions.

The initial step in a breeding programme with any crop is to accumulate a collection of diverse genotypes which may be used as source material for desirable genes. The germ plasm collections may include both local and exotic strains of the crops species and closely related species. While the breeder may collect local strains from the cultivator or from other breeders near by states.

In India, the introduction maintenance and evaluation of plant materials are vested in a division of plant introduction with headquarters at the Indian Agricultural Research Institute, New Delhi over 25,000 indigenous and exotic plant or seed collections have been made and explorations in India and surrounding areas, plant and seed materials maintained by the division are supplied to plant breeders in India and materials are exchanged with other countries. The import of living materials into India must be accompanied by a certificate of health to prevent introduction of new pests or disease, otherwise the material will be destroyed at the part of entry by plant protection and quarantine workers. New introduction upon being received are given on identifying number and information is recorded on adaptation, and characteristics in so far as available. These groups of materials have letters prefixed to the numbers. These groups and the prefixes are (a) E.C. exotic collections (b) I.C. indigenous collections and I.W. indigenous wild. It is, therefore, possible from the prefix to identify whether the plant is a local or introduced

strain and if a local strain, whether wild or cultivated.

After seeds or plant stocks of a crop are introduced they must be catalogued, made available to breeders interested in testing them, and maintained in viable condition so that they may be used again at some future date. Maintaining viable seed or plant stocks is particularly important since the world collections are the best reservoirs of plant germplasm available to breeders in the future. To evaluate these large collections of germ plasm for breeding stocks it is necessary to grow in various agroclimatic regions in order to determine where particular strains may be adapted, e.g. in India regional substitutions are being developed by the division of plant introduction representing regions in which crops adapted to different climatic conditions may be grown and evaluated, e.g. non-hardy tropical or semitropical types may not survive the rigorous of a northerly climatic zone represented are as follows:-

Temperate zone - Simla;
Arid zone - Jodhpur, Rajasthan;
Tropical zone - Kanya Kumari;
Mixed climatic zone - Amarvata

Commercial varieties of fields crop may originate from introductions by growing the variety as introduced enmasse, selection of desirable strains from the Australia, was found to be resistant to black and brown rust, moderately resistant

to loose smut, and to possess good yield and grain quality. Ridley is recommended and grown in several areas in India. As improved varieties adapted to specific local environments are developed fewer and fewer of the introduced varieties of standard crop will be superior to the local varieties already in use. However, the introduced varieties may possess genes of disease or insect resistance or other desirable features which can be transferred to adapted varieties by hybridization.

2.2. Selection

It is one of the oldest breeding procedures and the basis of cell crop improvement. It has been practiced since the earliest time that man began to cultivate the crop. The present status of our cultivated crops is largely the cumulative result of all the selection that has been practiced through many centuries. Essentially selection is a process, either natured or artificial, by which individual plant or group of plants are sorted out from mixed populations. The efficacy of selection is dependent upon the presence of genetic variability. Two methods of selection are practiced in breeding:

(a) mass selection

(b) pureline selection

2.2.1. Mass selection

If a group of similar individuals is selected and harvested and the seed is composited of more or less similar

and supposed true breeding genotypes. A variety developed by mass selection is generally more or less pure for those physical features which may be easily seen and used as the basis for purification. Such as presence or absence of awns, colour markings or maturity. But its component lines may differ in quantitative characters, such as yield, size or quality since small differences in quantitative characters cannot be visibly distinguished.

First year

Select a few to several hundred plants with similar phenotype, harvest and composite seed.

Second year

Grow in preliminary yield test comparing with standard varieties as check. If mass selection is used to purify and old mixed variety, the variety from which it was selected should be included as a check, observe comparative height, maturity, lodging disease resistance, yield, quality or other appropriate characters.

Third to Sixth year

Continue in yield test to determine performance and adaptation in comparison with standard variety as checks.

Seventh year

Start seed increase for distribution. When used as a method for breeding self pollinated crop plants. Mass selection has two weaknesses. It is not possible to know whether the plants being grouped either homozygous or heterozygous for specific dominant character. Since the homozygous plant will segregate in the following generation, phenotypic selection may need to be repeated. The environment in which a plant grows affects its development and appearance with mass selection phenotype is superior in appearance owing to hereditary character or to environment.

2.2.2. Pure-line selection

The theory of the pure line was established by a Danish botanist, Johannsen, in 1903. Johanneen conducted selection experiment with a mixed seed lot of the princess bean. A progeny descendent solely by self-pollination from a single homozygous plant is known as a pure line. A pure line variety is developed by increasing the self-fertilized progeny from a single, true breeding plant. A variety developed by pure line selection is more uniform than a variety developed by mass selection, since all the plants in pure line variety will be exactly alike. This is assuming, of course, that the plant originally selected is homozygous for all gene pairs, an assumption which plant breeders often make, but a condition

which is seldom, if ever, completely realized. Shining Mung No. 1 is an example of how a pure line selection may be made from a population of mixed genotypes. In this example the original population was an introduced or exotic collection of seed, but many of the old local or 'desi' varieties are comprised of mixtures of genotypes. Many varieties are developed by pure line selection from mixed population in the early stages of a breeding programme with a self pollinated crop.

2.3. Hybridization

Hybridization means crossing of the two individuals as two parents which are genetically different. Hybridization is one of the important methods of plant improvement. The main objective of hybridization is to combine in a single variety desirable qualities of two or more taxa. According to the genetic and phenotypic similarity and dissimilarity we can easily classify the hybridization into:

(1) Inter varietal
(2) Interspecific
(3) Intergeneric

In the hybridization method of breeding of self-fertilized crops two varieties are crossed, and plants in which are combined the desirable features of the parents are selected from the segregating progenies for increase and testing with

hybridization the best characteristics of the parent varieties may be combined into a single, true breeding strain.

In a cross between Pusa 4 and Australian Federation varieties of wheat, a strain was selected, N.P. 165, which combined genes for good yields and quality from Pusa 4 with loose smut resistance genes from federation. In a later cross between N.P. 165 and Kenya E 220, several strains were selected and later released as varieties, which combined genes for high yield, quality and smut resistance from N.P. 165 with genes for high resistance to black rust and to brown rust from Kenya E 220.

In addition to combining visible traits of the parent varieties by hybridization, it is also possible to select plants from the progency of a cross that will be superior to the parents in those features of the quantitative nature, such as yield, straw stiffness, or quality, in which inheritance is determined by multiple genes. These superior combinations, known as <u>transgressive segregates</u>. Many important improvements in plant breeding by hybridization came about by slowly accumulating desirable gene for quantitative character from diverse parental types. While the result may not always be as spectacular as when a single character, such as rust resistance, controlled by a single major gene is added to a variety, the progress in the long run may just as important.

In the hybridization method of breeding of self pollinated crops, the parent varieties are artificially cross pollinated. Artificial cross-pollination is relatively easy with grains which have large floral parts. The plants within the variety will be homozygous and identical. The F_1 plant although highly heterozygous, will have similar genotype and will look exactly alike. Genetic segregation will begin with F_2 generation, and heterozygosity will be reduced by one half with each succeeding selfed generation. The number of F_1 plants needed will depend upon the crop and the size of the F_2 population, from 1,000 to 10,000 plants, depending upon the similarity of the parent varieties and the number of characters from each parent varieties and the numberof characters from each parent that the breeder desires to combine in the progeny, will be needed to give a wide range of genetic segregation.

Chapter 3.

3. NEW BREEDING TOOLS

The methods of breeding already described deal largely with finding strains or plants with superior combination of genes in existing populations and improving these by selection into agricultural varieties, or with the creation of mixed genetic populations by artificial hybridization from which superior genotypes may be selected. It is by these conventional breeding methods that most new agricultural varieties have been developed in the past. The extent to which a particular crop can be improved by these methods of breeding is limited by the amount of variability within the species and its availability to the breeder.

In the evolutonary process which plants undergo in nature, gene recombination by natural hybridization plays an important part in increasing the variability within a species. Two other natural forces which increase variability are (a) mutation and (b) polyploidy.

The importance of mutation and polyploidy in the evolution of plant species has long been known. But it is only in recent years that means have been available for the practical plant breeder to create at will and utilize mutation or polyploidy for the development of improved agricultural varieties.

The knowledge that radiations and chemical mutagens will increase the mutation rate in a crop species has led to the development of a new breeding procedure. This procedure, sometimes referred to as mutation breeding, is yet in the developmental stage. The discovery that polyploidy can be artificially induced by use of colchicine and other means has stimulated the practical breeder to utilize variants created by doubling chromosome numbers, or by combing chromosomes sets from species hybrid as source of new breeding materials.

3.1. Mutation breeding

It has been known since 1928 that mutation may be induced in plants by various forms of radiation.

The common procedure with this type of study is to irradiate dry seeds with x-rays or atomic radiation known as thermal neutron or to treat with chemical mutagens. Treated seeds are generally reduced in germination, depending upon the reaction of the particular species and variety and upon the severity of the radiations. Seedling plants grown from the treated seed may vary from very weak to normal in appearance. The mutations are usually carried in sectors of plants in the generation following radiation so the R_1 plants are generally harvested by tillers or branches. The R_2 generation is then studied to find plants which have segregated desirable mutant characters. Some of the common mutations

observed which may be beneficial to the breeder include shorter straw, higher yield, larger kernels, early maturity, and disease resistance. Selected mutant plants are harvested and planted in progeny tests in the R_3 and later generation for evaluation of the mutant characters.

3.2. Polyploidy

Many crop species are natural polyploids i.e. their chromosome number has been increased by multiples of the haploid number. They include common cultivated crop species such as wheat, oats, cotton, tobacco and many forage grasses and legumes. Characteristics of natural polyploids are larger size, increased vigour and greater productivity. This fact has suggested to breeders the possibility of increasing the yield of plants of a particular species by artificially doubling or otherwise increasing the chromosome number. Artificial polyploids of almost all of the common crop plants have been produced at one time or another. In general, these polyploids are larger in size than the corresponding diploids, probably as a result of the increased cell size which generally accompanies the increase in chromosome number. Other changes in plant structure usually associated with polyploidy are thicker and stouter stem, broader and thicker leaves, and larger fruit and seed. Few of many of the newly produced or raw polyploids are immediately useful in agriculture. They possess certain defects which must be corrected by further breeding before

they become superior to the corresponding diploid. Different crop species differ in their response to polyploidy. Rye, red clover, white clover and sugar beets offer promise of being adapted to this type of breeding. Polyploids of soy-beans, linseed and maize, on the other hand, have been quite inferior. In general, plants with low chromosome number respond more favourably to chromosome doubling than plants with high chromosome numbers. Crops grown for their vegetative parts than for seed appear to be better suited for polyploidy breeding since chromosome doubling tends to increase plant size but has a deletarious effect on seed production. More success has been attained with cross pollinated crops than with self-pollinated crops. Since there are more possibilities of desirable recombinations with cross-pollinated plants. An example of successful polyploidy breeding is the variety of rye known as tetra-petkus. It was produced by doubling the chromosome number of a European variety. Tetra-Petkus rye is an exception to the generalization made above that crops grown for their seed are unsuited for polyploid breeding but does fulfil the other two requirements. The fertility of Tetra-Petkus rye is reduced if permitted to cross pollinated freely with diploid variety.

Another type of polyploid which has received much attention was derived from rye-wheat crosses known as Triticale,

the rye-wheat contains 42 chromosomes derived from wheat and 14 chromosomes from rye for a total of 56 chromosomes. Polyploidy has been used in some countries to improve the sugar beet. In sugar beets the triploid has been the most productive state of ploidy. Tetraploids of sugar beets are produced and crossed to a diploid to produce the triploid.

Chapter 4.

4. TECHNIQUES IN HYBRIDIZATION

In all fields of science the experimenter develops special skills, procedures, and techniques. In the practice of plant breeding the breeder finds or creates strains with desirable characteristics from the mixed populations and tests the selected strains in pure lines or in combinations to determine if they possess the characteristic for which he is looking in the desired intensity.

Selfing and crossing techniques

Selfing and crossing are essential procedures in breeding crop plants. It is important that breeders are master in these techniques in order that he may manipulate polination according to his needs.

4.1. Selfing

Selfing or inbreeding of self-pollinated species offers no particular problem to the breeder. In them, the plant is permitted to follow its normal modes of pollination and the seed is harvested. This is the procedure used with wheat, rice, barley, pulses, soybeans, groundnuts and similar crops when making plants or panicle selections. It is important that

the breeder knows something about the extent of natural cross-pollination within the breeding material.

In the selfing or in breeding of cross-pollinated species it is essential that the flower be bagged or otherwise protected to prevent natural cross-pollination. In the cross-pollinated species of grasses, which are pollinated by wind-blown pollen bagging the heads with parchment or glassing envelopes is a common procedure. It is usually necessary to shake the bagged heads daily until flowering is completed to disseminate the pollen. Seed set is frequently reduced in heads enclosed in bags probably because of the excessive temperature inside the bag. In crops like cotton which have large flowers the petals may be folded down over the sexual organs and fastended and these are pollen and pollen carrying insects may be excluded. Hand tripping in addition to bagging is generally necessary in many legumes to obtain self-pollination. In certain legumes which are almost entirely insect-pollinated, the plants may be caged to exclude the insects. In maize a bag is placed over the tassel to collect pollen, and the shoot is bagged to protect it from foreign pollen. The pollen collected in the tassel bag is then transferred to the shoot.

4.2. Emasculation

Knowledge of crossing procedure is extremely important to the plant breeder. Crossing is generally preceded by removing the stamens from the seed parent, a process known as

emasculation. The stigma is then pollinated with pollen collected from the pollen parent. Various techniques have been devised to facilitate emasculation and pollination.

Emasculation is unnecessary in monoecious or dioecious crops. With them it is necessary only to protect the pistillate flower before the stigma ripens and becomes receptive from foreign pollen until it is pollinated by the breeders with pollen collected from the desired source. With bisexual flowers, emasculation of the seed producing flower is completed before the anthers ripen and self-pollen reaches the stigma or emasculation is circumvented by some procedure that will permit an acceptable degree of cross-pollination. Some of the emasculation procedures commonly used by breeders are described below:

(1) Renoval of anther

Anthers may be removed with the aid of forceps, suction, or other means, before pollen is shed. This is the most common method of emasculation with wheat, rice, barley, oats, beets, tobacco and many other crops. Small forceps with thin, rounded points are desirable for soybeans, grasses and other crops with extremely small flowers. Fine pointed forceps or small bent hooks are sometimes used with small flowered legumes. Suction has been used successfully to emasculate small flowered legumes. A small pointed instrument or a lead pencil may be used to

roll out the anthers of linseed and sugar beets. Anther of tobacco may be plucked off by hand.

Killing the pollen by heat, cold or alcohol

Hot water has been used to kill pollen in sorghum, rice and grasses and thus the removal of anther is unnecessary. The flowers are immersed in hot water with temperatures ranging from 45 to 48 degrees centigrade for periods varying from one to ten minutes, depending upon the species. Chilling has been used with wheat and rice with temperature arounding freezing. Use of hot or cold water is a simple procedure since a thermos flask may be filled with water at the desired temperature and taken into the fields, and the flower may be immersed in the water for the necessary period of time. Hot water is used to open the flowers of rice, after which the anthers are removed with forceps. Pollen of inceme has been killed by immersing the flower in 57% ethyl alcohol for a period of ten minutes.

Pollination without emasculation

Self incompatible lines may be found in tobacco, potato, a few varieties of mustard, and many forage legumes. In highly self sterile plants emasculation may be unnecessary for the production of hybrid plants in which case the breeder depends entirely upon the greater compatibility of cross pollen to fertilize the ovule.

Male sterility

Genetic male sterility, conditioned by the presence of recessive genes has been used to eliminate the emasculation process in barley crossing. Cytoplasmic male sterility is used to facilitate the commercial production of hybrid seed in onion, maize, sorghum, bajra and other crop plants.

4.3. Pollination

Pollination must be made during the period that the stigma is receptive. This may be indicated by the opening of the flower and full development of the stigma. In some species such as rice, soybeans, cotton and tobacco, pollinations may be made on the same day that the flower is emasculated. In many species pollinations are usually delayed from one to three days after emasculation. Pollination is carried out by collecting ripe anthers and emptying the pollen from a dehiscing anther upon the stigma. The anthers are handled with five pointed forceps; or the anthers may be crushed and the pollen dusted over or rubbed on the stigma by means of forceps, toothpicks, small pieces of cardboard or hair brushes. In some cases the shedding panicle can be shaken over the clipped and emasculated florets. It is essential that the pollen be mature and fresh. Pollen collected from anthers, several hours before they would dehisces naturally, will give unsatisfactory result. In the length of time that pollen remain viable is

usually short. The pollen of maize may be killed in a few hours. With proper storage pollens of maize and sugar cane have been kept viable for several days.

Flowering of most crop plants occurs in the morning. So pollen is collected and pollinations made at that time. Oats flower throughout the day, and success is usually obtained by making the pollination in late afternoon. Pollinations are most successful when made on bright, warm days. Little is accomplished on cool, clowdy days.

Insects may be used to cross pollinate certain crops like mustard and incerne. The parent varieties are enclosed in an insect proof cage. Bees or other insects, which have been cleansed first of pollen, are introduced into the cage. A high degree of incompatibility is usually depended upon to prevent self or sib-pollination.

4.4. Practices to control flowering

Many crosses are made in glass houses during the winter months so that this tedious and time consuming operation will not fall at the period of optimum note-taking in the field. By making crosses of annual summer growing crops during the winter months, an extra generation is usually gained over the time required when the crosses are made in the field. Also, contamination from wind-blower pollen may be reduced by glass house pollinations. Glass house pollination often requires

use of various procedures. Procedures used include temperature control, regulation of day lengths and vernalization. Flowering may be speeded up by growing plants in higher temperatures in which the plants grow. Long day plants may be brought into flowering during the winter months by increasing the day lengths with artificial lights or by interrupting the period of darkness with a short period of light about mid-night. The same technique may be used to prevent certain short day plants from flowering prematurely.

4.5. Use of embryo culture with wide crosses

In interspecific and intergeneric crosses, it may be exceedingly difficult to obtain viable F_1 seed that will develop into a plant. In some cases it is possible by excising the embryo from the remainder of the seed and by culturing it aseptically on an artificial medium to obtain its germination and develop into a hybrid plant in difficult crosses such as barley sweetclover, fruit trees, forest trees and various vegetable crop plants.

4.6. Methods of breeding cross-pollinated crops

The methods used in the breeding of cross pollinated crop, or crops such as cotton and sorghum which have both cross and self-pollinated crops are not clearly defined as the method used in the breeding of self-pollinated crops. In addition, the methods tend to vary with the particular crop with which

the breeder is working. The methods of breeding hybrid maize are well adapted to that crop because the location of pollen bearing flowers in the tassel in maize makes possible and thus the production of hybrid seed on a field scale.

The principal methods which new varieties of cross-pollinated crops originate may be classified into four groups: (a) Introduction (b) Mass selection (c) Development of synthetic varieties and (d) Hybridization.

4.6.1. Introduction

Introduction may be used as source of new varieties as in self-pollination crops. Some varieties are grown as originally introduced. Introduction may also be used as source of desirable genes for disease and drought resistance, quality and other valuable characteristics, which may then be incorporated into adapted varieties by hybridization procedures, or which may be compounded into synthetic varieties.

4.6.2. Selection

Selection procedure used in breeding cross-pollinated crops differ from those used in self-pollinated crops. In the self-pollinated crop individual plants selections are used to establish a variety for the simple reason that segregation and cross-pollinated crops which are highly heterozygous

individual plants are seldom used to maintain the parent type within the progenies and a wider range of genetic diversity is generally needed to maintain a vigorous population. In cross pollinated fcrops, mass selection is a more common type of breeding than single plant selection. Selection procedure more commonly used with cross pollinated crops in addition to mass selection, include progeny selection, line breeding, and recurrent selection.

Mass selection

Mass selection is a selection procedure in which individual plant with desirable traits are chosen and bulked together to grow the following generation. It is based on phenotypic selection, that is on the appearance of the plant and on its particular traits that can be identified. The selected plants are harvested, generally without control of population, and are bulked without benefit of progeny testing.

The breeding progress that may be made by mass selection is limited into the range of genetic variability already present in the population. Since selection in naturally cross-pollinated crops is based on the material plant only, there is no control of the pollen parent or the gene it contributes to the progeny. Also it is not possible to distinguish between plants phenotypically superior owing to the environment and those superior owing to heredity.

Progeny selection and line breeding

Selection is a procedure in which progenies are grown in individual plots in order to determine the breeding behaviour of selected plants. By the progeny test plants whose superiority is due to genetic variation may be distinguished from plants whose superiority is due to the environment. In cross-pollinated crops individual plants are more or less heterozygous and the progeny will segregate for the heterozygous characteristics. Progeny selection is most easily carried out with crops that may be evaluated and harvested as individual plants, such as cotton, jute, sunflower. With progeny selection, open-pollinated seed may be harvested from selected plants, or pollination may be controlled in some manner so that selfed seed may be harvested. Selfing tend to fix character in a pure form. Since self-pollination leads to homozygosity.

Recurrent selection

Recurrent selection is used with cross-pollinated crops to concentrate genes for a particular quantitative characteristic in a population.

4.6.3. Hybridization

Two basic hybridization procedures are used in the breeding of cross-pollinated crop. These involve inter-varietal or interspecific crossing and utilization of hybrid vigour.

Intervarietal and interspecific crossing

Cross between varieties or between species, may be used to combine genes for desirable characteristics from different parents, as with self-pollinated crops. In cross-pollinated crops each plant may itself be an individual hybrid, in which case segregation will occur in F_1 generation. Hybrid plants in the progeny of the cross, if pollination is uncontrolled, will in turn cross freely with other hybrid plants within the population so that the progeny from the cross is not resolved into homozygosity as with self-pollinated crops. For this reason after hybridization selection procedures will differ from those used with self-pollinated crops. Phenotypically desirable hybrid plants will usually need to be selfed for more generations to fix the desirable character in a homozygous condition. From the hybrid population, by progeny selection, lines are then established which combine the desirable characteristics of the parent varieties. Some form of outcrossing the selected lines eventually may be necessary to restore the vigour lost during inbreeding.

Utilization of hybrid vigour

It is common observation that the F_1 generation in many crosses is more.

Chapter 5.

5. TECHNIQUES OF INDUCTION OF POLYPLOIDY

5.1. Methods of treatment with colchicine and its results

A plant like an animal owes its hereditary nature to the chromosome that it contains in each of its cells. Any change in the chromosome of an individual is transmitted to its offspring and subsequent generation. One of the possible changes is a simple, complete doubling of the chromosomes sets of a group of cells.

The idea bore fruit in several quarters almost simultaneously. A number of reports on the cytological effects of colchicine on plants appeared in 1937 and 1938. Papers by Blakeslee, Avery, Nebel and Ruttle established colchicine treatments as a relatively simple and reliable means of inducing chromosomes doubling at will.

Since colchicine acts on cell only while they are in the process of division to be effective it must be supplied to actively growing regions that contain a high proportion of dividing cells. Consequently germinating seeds and actively growing stem tips have been the subject of much experimental work.

Treatment of seeds

One of the simplest methods of treatment is to soak seeds prior to germination in a 0.05 to 1.5% aqueous solution of colchicine for a period of 1 to 6 days. Seeds that germinate slowly require longer treatment, but factors other than germination time, as yet unknown, have also played a part in the seed-soaking treatment. Results from this method have not proved entirely satisfactory for in many instances germination has been retarded or the percentage of germination has been reduced. Several experiments have been reported in which treatment of seeds failed whereas treatment of seedlings or of buds of the same species succeeded. On the other hand, a slight stimulation of growth has been reported for corn cabbage and mungo bean as a result of soaking in concentration below 0.01%. Germination of petunia seeds was improved by soaking in concentration of 0.01 to 0.1%; only in concentration of 0.1% or above was the number of surviving seedlings much reduced.

Treatment of seedlings

Treatment of the stem tips of young seedlings gives more consistently satisfactory results than pregermination treatment of the seeds. Since young roots are easily injured by colchicine, it is better to treat the shoots separately than to expose the entire young seedlings to colchicine solution.

For ease in handling seeds are more commonly germinated on moist filter paper before treatment. Colchicine can then be applied to the young seedlings in any one of several different ways. If the seedlings are firmly attached to the filter paper by their roots, the paper plus seedlings can be inverted over a smaller pan of colchicine solution in which the shoots are immersed.

Seedling can be removed from the filter paper, rolled loosely in bundles, root ends wrapped in moist cotton and the plants inverted in a vessel containing colchicine solution so that the shoots are immersed. This method prevents the roots from drying out, yet keeps them from contact with the colchicine solution.

Seedlings can be removed from the filter paper and placed in a dish that has been divided across the middle by a ridge in such a way that the roots are immersed in water on one side of the ridge and the tops are immersed in colchicine solution. On the other side of the ridge long spindly seedlings that have grown in the dark arebetter adapted to this method than shorter stockier ones.

To offset the inhibiting effect of colchicine on the growth of seedlings, roots. Root inducing harmones have sometimes been applied after colchicine. Both favourable and negative effects are reported from such after treatment.

5.2. Treatment of older plants

Either individual stem tips or entire young shoots of

older plants can be treated. The following methods have been devised for treatment of individual branch tip. Each of them has been reported effective with one kind of plant or another.

Immerse a growing tip in a water solution of colchicine. This method can not be used with plants such as the potato that will not survive after immersion in water.

Prop a bit of absorbent cotton between the youngest leaves and keep it moistened with the colchicine solution.

Drop or brush the colchicine solution on a shoot several times a day for several days. Glycerine may be added to retard drying. A wetting agent such as santomerse will ensure close contact of the solution with the surface of the plant. Dermen recommends the following solution.

Apply colchicine in agar, either in a solution (0.7 to 1.0% agar in water) that can be brushed on the or in a gel contained in a drug capsule that can be placed over the bud.

Colchicine can be applied to whole leafy shoots as a spray. Warmke and Blackslee give direction for preparing an emulsion which they apply as a spray. However, spraying is dangerous and the preparation of the emulsion is quite difficult.

5.3. Caution

Whatever method of application is used it must be remembered that colchicine is a poison. It should not be allowed

to remain on the skin. Cook describes an experience in which colchicine accidently got into the eyes and caused violent inflamation and temporary blindness. When swallowed, it causes severe gastrointestinal disturbances with symptoms resembling those of arsenic poisoning. A large dose can cause death.

The effect of colchicine on cell division is highly specific and consists of inhibition of the spindle mechanism. Thus, the early stages of mitosis proceed as usual through the splitting of each chromosome into two. The colchicine effects enter at this point. Instead of the chromosomes arranging themselves in an equatorial plate and then separating into two exactly similar lots between which a new cell wall is formed, the chromosome remains for sometime in haphazard arrangement, then go back in a resting stage. They constitute a nucleus which now has twice as many chromosomes as before. If exposure to colchicine is prolonged beyond the duration of one mitotic cycle to the same doubling process may be repeated once or several times resulting in octoploid or higher ploid cells.

Sometimes the chromosome doubling effected by colchicine is not exact and the resulting nucleus do not have exactly twice the diploid number.

Colchicine is the chemical that has most useful in producing new varieties of plants. It is expensive but only small quantities are necessary. It can be applied in water

solution to buds or to young growing stem tips by any of several different methods. The proportion of useful new kinds of plants obtained by colchicine treatment is not high, but an occasional new form may more then justify much apparently fruitless work.

5.4. Special techniques for studying the action of colchicine

Pollen grains that can be used for artificial culturing work serve well for testing the action of colchicine upon mitosis and growth processes. The specific morphology of chromosomes were studied in polygonatum and discovery of natural polyploidy was made directly from these observations. Another valuable feature is that the small amount of chemical can be tested. Other mitotic poisons soluble in water can be adapted for testing with the pollen tube methods. Colchicine was used as effectively with root tips of Allium cepa that the test has become known as a method for experimental work, the Allium cepa test.

5.5. Chromosome studies

The pollen mother cells stained by acetocarmine are universally a most important source for studying chromosome in plants. The procedure for determining the number of chromosome is rapid. More important than deciding what the number might be are the pairing characteristics at meiotic metaphase configuration due to translocation and the irregularities of meiotic processes generally. These are the problems

associated with polyploidy that must be studied at the pollen mother cell stages.

Root tips are used for a check of the somatic numbers of chromosomes. Pretreatment of root before fixation with chemicals that mitosis at metaphase facilitates the study.

Pollen tube cells that undergo mitosis in the tube rather than inside the pollen grain can be treated with colchicine in sucrose-agar-media. Scattered chromosomes are easily counted and the morphology of somatic chromosomes in haploid sets can be measured.

5.6. Technique of colchicine application

The following facts and factors form some of the most important bases of colchicine polyploidy technique:

1. Cochicine in aqueous solution is obviously diffusible into plant tissue otherwise no internal changes could occur in meristematic cells as a result of surface application.
2. Dormant tissues are not so affected by colchicine as to result in polyploidy. Such results are obtained only in tissue where cell division is active. For practical result, treatments should be applied to tissue that will develop into vegetative sexual or both types of plant parts.
3. It is most important to provide and maintain the best cultural conditions for the treated material in order to obtain maximum results. It is especially important when

material is to be immersed in colchicine to keep the solution at an optimum temperature in order that cell division may not be impeded.

4. Duration of treatment is an important factor and should be determined for each type of material. It is dependent on the time required for the cycle of cell division in the particular tissue. It has already been shown that the effect of colchicine is not confined to a limited number of cells at a particular stage of cellular development such as metaphase.

Any cell may be affected which obtains colchicine by diffusion if such a cell goes through division while containing the chemicals. Furthermore as long as colchicine remains present in the treated material above a threshold concentration the affected cell will repeatedly fail to divide at the end of each chromosomal divisional cycle, resulting in multipolyploidy or multinucleate cells; consequently, successively affected tissue may often fail to survive. Undoubtedly the failure of growth and eventual death in treated seedlings and treated growing points of the shoots are often the results of the above factor. In order to avoid such excessive result, it may be necessary to estimate approximately the optimum time limit of the divisional cycle of cells of tissue that is required for the change into polyploidy in the treated material.

Chapter 6.

6. TECHNIQUES OF INDUCTION OF MUTATION

Since the proposed programme of research includes induction of mutation with chemical mutagens, some aspects of the techniques of treatment with chemical mutagens are described below.

6.1. Chemical mutagens

As early as 1943 a mixture of ethyl urethane and potassium chloride were used to induced translocation in Oenothera. Auerbech and Robson used sulphur and mustered gas on Drosophila and Gustoffson used on barley. Mustered gas induced in barley Albino and viridis mutents, colchicine was used for inducing in sorghums other plants also. Days to heading and height of plants were some of the characters affected by colchicine. Now a days most important chemical mutagens used are:

EMS (Ethyl methane sulphonate)

DES (Diethyl sulphonate)

Caffein

Creosoles

Meta nitrophenol

Mustered gas.

Sometimes chemical mutagens are used together with physical mutagens. This is called recombinad treatment.

The chemical mutagens are used either on seeds or on embryos. And the seed may be dry or previously soaked. The wet treatment of seeds is more effective than the dry treatment. The dosage required for dry seed. There are energy absorbed by the tissue is calculated in Kr unit which is equal to energy absorbed by 1 gm of tissue = 93 ergs. Wet seeds given 2000 kr. while dry seeds are given 4000 kr. O. K. Jain treated some seeds of chrysanthemum and obtained large number of mutations e.g. flowers with large number of petals obtained from single whorls.

E.M.S. can be used in concentration of 0.2 to 0.6% in double distilled water and DES in concentration of 0.2 to 0.8%. The soaked seeds are treated for 6 to 48 hrs. for desired effect. While treating the seed with mutagenic solution they should never submerged completely in mutagens.

While treating with chemical mutagens the ph of solution also should be controlled and that is why buffer solution of dibasic sodium phosphate or monobasic sodium phosphate are used. Phosphate buffer maintain the ph of the medium. Stock solution prepared and kept for use. The effect of chemical mutagens as the seed is reduced percent of germination reduced. Per cent of survival on seedlings shows some abnormality morphologically variations occur in cotyledons. Standard growth of seedlings, the change of leaf shape and pattern appearance of spines, delaying of the flowering period appearance of floral abnormality, effect on pollen fertility or a few of the important effects.

6.1.1. Preparation of phosphate buffer stock solution

With the treatment of embryos with phosphate buffer solution, first it is prepared.

(a) 0.2 m. solution of monobasic sodium phosphate i.e. 27.8 gm in 1000 ml.

(b) 0.2 m. solution of dibasic sodium phosphate i.e. 53.65 gm solution hydrogen phosphate or 71.7 gm of $Na_2 HPO_4$ in 1000 ml.

(c) X ml of (A) and Y ml of (B) is diluted to 1000 ml proportion of different values.

Soln. A.	Soln. B.	Ph
93.5 ml	6.5	5.7
92.0 ml	8.0	5.8
90.0 ml	10.0	5.9
89.7 ml	12.3	6.0
85.5 ml	15.0	6.1
81.5 ml	18.5	6.2
39.0 ml	61.0	7.0

6.1.2. Treatment with phosphate buffer stock solution

Instead of distilled water, seeds are soaked in phosphate buffer solution for 24 hrs. For this a filter paper is put in a petridish and the seeds are spread over the filter paper and then the buffer solution is poured until it partly covers the seeds. The soaking is done for required period.

6.1.3. Preparation of 0.6 - 8 EMS in phosphate buffer solution

To 99.4 cc of phosphate buffer solution added 6 cc of

EMS of the solution. For 0.8% EMS solution take 0.8 cc and add to that 99.2 cc of phosphate buffer solution.

6.2. Treatment of material with mutagens

A number of attempts have been made to induce mutation by physical or chemical mutagenic treatment. It is the seed and embryos which have usually treated and it has been found that in case of chemical treatment the effect produced in embryos its treatment is more as compared to seeds. It is because the embryo in case of seed does not come in direct contact with the mutagens. Here there are two ways:

Wet treatment

In this method the seeds are soaked in water for few hours before treatment.

Dry treatment

When the seeds are not soaked in water before treating with mutagens and it has been found that effect produced in wet physical treatment of mutagens are more than in the dry treatment. It is because when soaked seeds are treated, it contains some water. So more H_2O is produced which will cause visual change but in dry seeds the water is absent so the effect produced is less efficient. Moreover the dose of mutagens required in wet treatment is half that of dry treatment.

In chemical treatment there is not much difference in mutation rate whether it is dry or wet treatment because chemical mutagens itself contain water. Furthermore if embryos have to be treated the dry and wet treatment has not much different rate of mutation when embryos have to be treated with the mutagens. There are following difficulties:

Excision of embryos without injury.

Putting in culture tubes i.e. inoculation.

Maintenance of external factors.

Prevention from contamination.

Konzak showed that the effect of EMS treatment was lethal, if the seeds were dried but studies by Froese Gerbzen et. al. showed that the damage caused by drying barley seeds after EMS treatment was reduced considerably by soaking the treated seeds in distilled water before drying.

6.3. General method used

Spread the seeds in petridish and put some chemical mutagens. Keep in this for 6 to 48 hrs. depending upon the concentration of mutagens. If concentration is more the time of soaking is reduced. In 1 gm of water usually 1/2000 ions are present, e.g., in barley and Vicia faba etc.

Chapter 7.

7. TECHNIQUES IN CYTOLOGY

7.1. Collection and fixation of material

The killing and fixing of the material to be made into permanent preparations are important processes. Fixation is a process very difficult to describe in terms that are easily understood. It is commonly said to be the preservation of all cellular and structural elements is as nearly the natural living condition as possible or to put it a little differently, a good fixative is one that changes the cell chemistry the least and preserves the cell structure the best. What one actually seeds after fixation is always a picture entirely different from the picture during the living condition, as it is utterly impossible to preserve anything in the exact condition in which it existed during life. Fixation is thus an entirely empirical process. The success or failure of fixation is judged by the quality of its usefulness when the completed preparation is examined.

Fixation is required in order that structure, which are obscured or entirely invisible when cells are observed in the living condition, may be seen more distinctly and in order that soft structure may be hardened sufficiently for further treatment. There is a sharp difference in the final picture of the effects of fixatives on fixed structure and on the nonfixed structural elements of protoplasm.

Originally the term fixation was applied to the grosser structure which can be preserved without any relation to the change of the colloidal state which the fundamental structure

of protoplasm.

Bhaduri and Ghosh (1954) have described their method of chromosome squashes in cereals as follows.

Root tips were soaked 1-2 hrs. at 18-20° C in a freshly prepared saturated aqueous solution of L-bromonaphthalene, 1-2 hrs. in water and fixed in a mixture consisting of 1% chromic-acid, 5 ml.; 2% osmic acid, 1 ml; and 0.002 m 8-oxy-quinoline, 1 ml for 0.5-1.00 hrs. at 10-40° C. They were then successively treated as follows:-

Water 1-2 minutes 1% H_2SO_4, 10-15 minutes water 3-5 minutes 1 NHcl 45 minutes at 60° C water 3-5 minutes. Lencobasic fuchin 0.5-1.0 hr. The brightly stained tips of the root were cut off and squashed in the usual manner in a drop of acetocarmine under a cover glass. The cover glass was sealed with paraffin was removed and the cover glass separated in a 1 : 1 mixture of acetic acid and n-butyl alcohol. Final processing consisted of passing the slide through pure n-butyl alcohol and covering in balsam.

Narayan has described a technique for cytological preparation following enzymatic digestion of pollen mother cell wall.

A technique is presented for making meiotic chromosomes preparation with enzymatic digestion of pollen mother cell walls with cellulose. The technique has given excellent chromosomes definition from early pachytene stage on such chromosome preparation were found useful in situ nucleic acid hybridization studies on plant chromosomes.

7.2 Preparation of Acetocarmine stain

Really superb results have been secured by the use of this method. Much care is required to obtain successful preparations and the method is not applicable to every organism.

There are several methods of using iron acetocarmine. The method to be used with a particular type of material depends upon the material itself.

When viewing iron-acetocarmine preparations under the microscope, a green filter should be inserted between the microscope and the source of illumination. The bluish red chromosome will then appear nearly jet black. It is a common experience that plants with small chromosomes give poor results when stained with acetocarmine owing to the readiness with which the cytoplasm takes up the stain thus preventing adequate differentiation between chromosomes and cytoplasm. The staining reaction, as pointed out by Thomas (1940), depends on the following four factors which can be varied independently.

1. Constitution of the prefixative
2. Duration of fixation and storage
3. Strength of the stain and
4. Amount of iron introduced.

Numerous schedules based on altering these factors and aiming at greater standardisation, have been suggested by different authors to suit the requirements of different crops (e.g. Marks 1952, Hyde and Gardella 1953; Swaminathan 1954).

1. Fix the anthers expected to contain the desired stages of microsporogenesis in a mixture of 3 parts of absolute alcohol and 1 part of propionic acid saturated with ferric acetate. Fixation at low temp. for 24 hours gives good results. The material can be kept in the fixative at low temperature for a few months. The ferric acetate-propionic acid component of the fixative is best prepared once a week.

2. Place the anther in a drop of propiono-carmine on a slide. Smear it in the usual way. Judicious warming over a flame aids both proper differentiation and spreading. Use a plated needle to make the smear otherwise the staining becomes very dark owing to the addition of more iron through the needle.

3. Press the cover glass with a piece of blotting paper thus removing all the excess stain. Seal the slide with paraffin wax. The slide can be made permanent by any of the usual methods. The schedule used by Bhaduri and Ghosh (1954) gives satisfactory permanent preparations. In this method, the slide is inverted in a mixture of

glacial acetic acid and N-butyl alcohol (1 : 1) till the cover glass separates. The slide and cover glass are then passed through N-butyl alcohol (one minute) and then mounted in neutral balsam.

The method adapted for preparing propiono-carmine is as follows:

Carmine in larger excess of what will dissolve (about 2 gms per 200 cc) is added to boiling 45% propionic acid and boiled until there is a sudden change to darker colour. The flame is removed as soon as the change in colour is noticed. The stain is cooled and filtered. The use of propionic acid in both the fixative and stain in place of acetic acid which is mor often used in such schedules gives better result probably becaus it hold more iron in stable solution and also dissolve more carmine.

7.3. Preparation of slides with smear or squash techniques

The more refined of the various smear methods afford, in a relatively short time preparations which are of the greates value in counting the monoploid and diploid chromosomes and in studying the chromosomes themselves. In fact, smear preparation are so useful that at the present time many critical cytological studies are based principally upon such slides. The smear metho is limited to some extent in applicability to cells which are no firmly united to one another, as by middle lamellae.

The essential idea underlying all smear methods is to spread the cells out into a single layer in order that they may be killed instantly and fixed evenly and uniformly, without distortion or the production of artifacts. Practically all cells which can be smeared will adhere to the slide, hence the necessity of using a cementing agent is obviated. The slips can be carried through the various staining and dehydration process with the loss of only a few cells provided, of course, that the changes are not too violent.

The slides upon which the smears are to be made must be chemically clean. New slides should be given a long immersion in the sulphuric acid-potassium bichromate mixture. rinsed in running water, placed for a short time in strong alcohol to which a little ammonia is added, rinsed again, and finally dried with an absolutely clean cloth free from starch and lint.

The fluid which is generally considered to be superior to all others for the killing and fixing of smears is Navashins. The two portions composing this fluid are mixed together just before using. It may be necessary in some cases to vary the proportions of some of the ingredients, particularly the acetic acid.

Some technicians have methods of making smears adapted to their own needs. Most of them remove the anthers from the buds, cut off both ends, or cut each anther into segments if

it is over 2 mm. in length, and place near one end of the slide with a grease pencil in order to that one may know on which side the smear is made. With a clean scalpel, which should be honed flat and smooth, quickly and evenly crushed and spread the anthers over the centre of the slide so that the microsporocytes are in a single layer over the greater part of the slide and place in the petridish in such a way that the entire smeared surface comes into intantaneous contact with the killing fluid. If the slide is brought into the fluid in any save a perfectly horizontal position, most, if not all, of the material will be washed off. The time elapsing from excising the anthers to placing in the killing fluid should not exceed 4 seconds. As the capacity of petridish have two slides, a second smear may now be made using anthers from another bud if desired, and placed beside the first. Although the slides may be turned smear side up after about 10 minutes. It will be safer to leave them as they are for the duration of the time necessary for proper fixation, which with Navashin's fluid is about 4 hrs.

Another method of smearing the anthers or pieces of anther is to place them in the centre of a slide to hold a second slide crosswise, and to exert just enough force firmly to extrude and to spread the microsporocytes. Immediately invert both slides in the dish containing the killing fluid.

To the mixture add approximately 1% saponin, the amount may have to be adjusted for different species. The fixation completed, turn the slide right side up and with forceps remove anther fregments and other thick pieces of debris that might unduly elevate the cover slip when mounted. Thus place in low staining dishes and washed about 15 minutes in gently running water. The slide should be examined under the microscope and any undesirable once rejected. The smears are now ready to be stained.

Squash and smear methods have dealt with tissue that have been sectioned. Other means are used to handle types of materials. One of these are the squash or smear method, used primarily for chromosome studies.. In this method a naturally soft piece of tissue is pressed between the slide and the cover slip. This results in mechanical separation of the cells. The cells are then stained usually by using dye that colour only the chromosomes. The resulting preparations frequently show with starting clarity chromosome detail not readily seen used in chromosome counting work. Since all the chromosomes of the cell are visible in the same field. Two famous and widely used stains are for smears and sequashes. These are the acetocarmine and Feulgen stains. The third less generally used is the iodine crystal violet stain, which although not yield fine preparations. The acetocarmine method developed by Belling (1926) for chromosome work, combines the killing, fixing and staining solutions. In its simplest form

the procedure involves merely placing the fresh tissue on the slide with an iron needle, placing a cover slip over the preparation to squash the tissue flat heating the slide gently with an alcohol lamp, and examined it under a microscope. The chromosome appear dark, red the cytoplasm, either pink or colourless. Tissue fixed in alcohol-acetic acid (3 : 1) may be studied.

The acetocarmine solution consists of 1% of carmine in 45% acetic acid. The iron needle that is used to tease the tissue apart add a small amount of iron. The fixation is provided by the acetic acid and excellent for chromosome. The carmine acts as a basic dye, and the iron may be involved in some way as a mordant. Orcein may be substituted for carmine and stains some chromosomes better.

The ease of obtaining good preparations with the aceto carmine method has made it one of the most widely used staining procedure for chromosomes.

Squash preparations are usually temporary in nature unless given further treatment. This treatment may consist of removing the cover slip from the slide dehydrating the cells, and then using a permanent mounting medium to remount the cover slip. Other method can be used for making permanent mounts. These methods involve moving materials under the cover slip. One such method is given in the section on procedures. Chromosomes

frequently require special pretreatments and other refinements of techniques to yield the data required for such special method as well as for an excellent treatment of chromosomes techniques.

7.4. Cytological methods

Microtechnical methods employed in the field of cytology are the most critical of all, and before a person enters this domain, he should take the pains to become fairly well grounded in the basic principles and procedures of general botanical microtechniques. Only in this way it is possible to become sufficiently to devise the special variations in schedules demanded by the differences in structure and chemical composition of the tissue of diverse plants. No two species react exactly alike to the identical technical schedule, and the beginning technician must learn how to adapt the composition of killing and fixing fluids and the staining procedure to the particular plants under investigation. The common practice of attempting to learn microtechnique and to investigate a cytological research problem simultaneously cannot be too strongly condemned.

Cytological technician in the past have been too content to adapt pragmatism as their sole philosophy. The rational of their methods has too often mattered little or not at all, provided the final result was according to their notions.

Chapter 8.

8. BREEDING OF WHEAT

As example the work on wheat has been described. Wheat is the leading grain crop of the temperate climate of the world, just as rice is the leading grain crop in the tropics. Although cultivated under a wide range of climatic conditions the most extensive production of wheat is in areas where the winters are cool and the summer comparatively hot. In South and Southeast Asia cultivation of wheat is concentrated in Central Northern and North-western India.

8.1. Pollination in wheat

Wheat is a self-pollinating crop. Flowering begins in the upper part of the spike and throughout the day with two to three days required for a spike to finish blooming. The glumes normally open during the flowering process. The anthers protrude from the glume and part of the pollen is shed outside the flower. Entry of foreign pollen while the flower is open may result in a small amount of cross-pollination. Normally cross-pollination is less than 1%. If conditions are unfavourable for the opening of the glumes the anther may shed their pollen without being extruded. To exclude all cross-pollination in breeding or genetic studies the spike may be covered with a butter paper envelope prior to flowering.

When two varieties are to be cross-pollinated flowers from the variety to be used as the female parents are emasculated

and then pollinated with pollen collected from the male parent variety. Wheat flowers are emasculated on the day prior to their shedding of pollen by clipping back the glumes and removing the anthers with fine-pointed tweezers. Pollinations are made one or two days later by breaking a ripe anther over the stigma. Crossing may be facilitated by the utilization of male sterile lines, thereby eliminating the emasculation process. Genetic male sterility and cytoplasmic male sterility are both available in common wheat.

8.2. Methods of breeding wheat

Systematic and organized research on the improvement of wheat in India by breeding was initiated by the late Sir Albert Howard and Mrs. Howard at the agriculture research institute.

New varieties may originate through (a) introduction (b) selection (c) hybridization. These methods of breeding self-pollinated crops were described.

8.2.1. Introduction

Introduction did not play an important part in the early breeding of varieties in India and Pakistan. Foreign or exotic varieties imported by Howards were mostly found to be too late in maturity for Indian growing conditions. Introduction have been used extensively in wheat hybridization programmes in India and Pakistan. Through hybridization the resistance genes have

been transferred to adapted types and new varieties have been developed which combine the yield and quality of the Indian wheats.

Recently several short strawed varieties have been introduced from Mexico which may greatly change the wheat variety pattern in India e.g. Sonara 63, Sonara 64.

8.2.2. Selection

It has already been mentioned that when the Howards started wheat improvement work in India at Pusa they had little success at first with foreign introduction, either used directly as varieties or as parents in crosses since most introduced varieties were too late in maturity for the Indian climate. However, the Howards were able to develop within a short time several varieties outstanding in yield and quality, by selection from local types.

In the Punjab, varieties selected from local types included Punjab types of distributed in 1911, Punjab type 11 distributed in 1913 and Punjab type 8A distributed in 1919. Some of the other varieties selected from local types were Kanpur 13 and C.46.

8.2.3. Hybridization

Since about 1925, most of the important varieties of wheat developed in India have been developed by varietal hybridization. After the superior selections had been isolated from the original local's type, it became apparent that important advances could

not be made except by hybridization. This sequence in obtaining new varieties is logical, for an intelligent hybridization programme can be developed only after the parent materials have been sorted, tested and the best strains among them have been identified. Also, the large accumulation of knowledge in the field of genetics during the early part of this century made possible a clearer understanding of the mechanics and principles involved in combing the desirable characteristics of parent varieties through hybridization. However, the practice of hybridization had been practised much earlier as the following examples will show. The Fulcaster variety of soft red winter wheat was produced in the U.S.A. in 1886 by a farmer breeder, S.M. Schindel in Maryland from a cross between Fultz and Lancaster. This variety was widely grown in the U.S.A. until a couple of decades ago.

8.3. Interspecific and intergeneric crosses

In interspecific and intergenetic cross involving common hexaploid wheat species at the tetraploid level may be used to transfer desirable genes, such as rust resistance, insect resistance, and other characters from tetraploid species to common wheat. In the U.S.A., Stem rust resistance genes were transferred from Yaroslavmmer to the Hope variety and from lumillo durum to the spring wheat variety. An intergeneric cross involving the use of X-ray to assist in the transfer of a leaf rust resistance genes from the diploid species.

8.4. Mutation breeding

Radiation and chemical mutagens may be used to increase mutation frequencies in wheat as in other crops. The most common observable mutations following radiations have been speltoids, compactoids, sub compactoids own mutation, chlorophyll mutation and other abnormalties undesirable to the breeder. These may generally be classed as macromutation and are often accompanied by sterility and other undesirable pleiotropic effects. In addition, many small micromutations occur the effects of which are not visible in single plant but can be measured in a population of plants. The micromutation may be more useful than macromutations in breeding since they are less likely to be accompanied with pleiotropy or sterility. Sterility following radiation may result in outcrossing if plants are unprotected from foreign pollen offtype plants selected from advanced generations of iradiated populations may therefore be a result of mutation plus outcrossing.

Two varieties of wheat, lewis and stadler, have been developed at the Missouri Agricultural Experiment Station in the U.S.A. by mutation breeding. Both varieties were selected following irradiation with thermal neutrons of an improved experimental strain. Lewis is shorter and stiffer-strawed and stadler is higher in yield and test weight and has more resistance to leaf rust than the parent strain. It is not known whether the change observed are the direct result of alteration in the genic

alterations and outcrossing have both occurred. The latter appear to be the most plausible explanation. The two varieties were named after Dr. Lewis J. Stadler. Irradiation with X-rays was utilized at the Missouri Agriculture Experiment Station in the U.S.A. to obtain a cross over between a wheat chromosome and an alien chromosome from Agilops.

8.5. Hybrid wheat

Heterosis in yield and other character in wheat have been observed for many years. Utilization of hybrid vigour as a method in breeding wheat become possible after finding cytoplasmic male sterility and pollen fertility restoring genes in the wheat pollen thus permitting natural cross-pollination instead of self-pollination as normally occurs. Pollen fertility is restored to the hybrid wheat by dominant fertility restoring genes contributed by the pollen parent. Breeding and utilization of hybrid wheat involve three steps (a) development and maintenance of male sterile lines (b) crossing of male steriles with fertility storing lines and (c) utilization of these in the commercial production of hybrid seed.

Hybrid wheat is the F_1 progeny of crosses between two selected parent lines. In the production of hybrid wheat one parent will be the cytoplasmic male sterile. A line produced by the procedure described above, the pollen parent, known as the R line will be one that (a) restores fertility in crosses with the male sterile A line and (b) nicks with the A line to produce a vigorous and productive F_1 hybrid. The fertility restoring

genes may be transferred to a wheat variety to be used as R line by the back cross procedure. Male fertility in cytoplasmic male sterile wheats with T. timopheevi cytoplasm male sterile wheats with T. timopheevi cytoplasm may be restored by the presence of two dominant genes, Rf, and Rf_2. These genes are present identified in a plant selected from the cross T. timopheevi Marquis. Presumably these genes the T. timopheevi for without these wheat would not produce fertile pollen.

In the production of hybrid wheat the A line contains sterile cytoplasm and recessive genes for fertility restoration. Hence it is male sterile. The B lines contain recessive genes for fertility restoration but has fertile cytoplasm, hence it is male fertile. The R line will have the dominant gene for fertility restoration, Rf_1 and Rf_2, but may have either sterile cytoplasm or fertile cytoplasm, in either case it will be male sterile. It may be easier to develop restorer lines with sterile cytoplasm than with fertile cytoplasm. Since the presence of the Rf restorer gene will be apparent than without test crossing into male sterile lines. The cytoplasmic and genic content of the lines utilized in breeding hybrid wheat with the T. timopheevi cytoplasm.

In the beginning of the hybrid wheat programme standard varieties or experimental lines will be converted either to male sterile A lines or to fertility restoring R lines and used in the

production of hybrid wheat. Present evidence indicates that yield increases in the magnitude of 25 to 30% over the average of the parents may be expected to be increased as research advance and new lines are developed with superior combination ability. Current research indicates that 50 to 60% seed set in seed production for pollination with a ratio of 1 male pollinator row to 2 female hybrid seed producing rows. More experience is needed in seed production procedure to determine finally the ratio of seed producing to pollinator rows to plant The condition for obtaining maximum seed set and other details which will affect the economy of hybrid wheat seed production. Experience is needed to learn which varieties may be converted into male sterile A lines and which may be used as fertility restoring lines. Since it will not be possible to convert varieties with male fertility restoring genes to male steriles, the latter presumably may be converted to male fertility restoring R lines. Much breeding and testing is also required to develop and identify the lines that will combine to produce high yielding F_1 hybrids with acceptable agronomic type and backing quality. It is also necessary that the A lines and the R lines flower at the same time in order that cross pollination is affected.

Here are some selected examples of work on wheat: Sears (1944) conducted cytological studies with polyploid species of wheat with reference to additional chromosome

aberration in Triticum vulgare. Seventeen of the 21 possible nullisomics in Triticum vulgare var. Chinese Spring have been obtained, 11 of these involve chromosomes homologous to these of the emmer or tetra ploidy wheats. The location of several genetic factors revealed by nullisomics.

Nullisomics have been recovered in frequencies of less than one to more than 10% in the progenies of monosomics. Tetrasomics have occurred among the offsprings of trisomics in frequencies of about one to ten percent. Telocentrics and isochromosomes occur fairly frequently among the offsprings of monosomics plant, following mid division of univalent chromosomes Goud (1966) studied induced mutation in bread wheat. To evaluate the efficiency of treatment with gamma rays, fast neutrons and EMS in producing mutations in qualitative characters in bread wheat and experiment was conducted with six varieties (N.P. 876, N.P. 872, N.P. 870, N.P. 863 and N.P. 862) treated and control seeds of all the six varieties were sown in the field The growth and survival was much hampered in EMS treatment, 30 kr gamma irradiation, N.P. 870 was much less affected by EMS treatment as was relatively more resistant to gamma irradiation. About 10 and 35% of chimeras were observed in gamma and EMS treated plants respectively in M_1 generation. The spectrum and frequency was very high in EMS treatments followed by gamma irradiations. The mutation frequency varied with variety

N.P. 870 was highly mutable whereas N.P. 862 was rather stable with the other varieties falling in between gamma irradiation produced more of albino and speltoid mutations whereas EMS produced more of Xantha, viridis sub compactoid, compactoid and sphaero-coccoid mutations. The mutation spectrum was considerably increased in EMS treatment. Prasad, Joshi and Swaminathan (1972) have worked on mutation and recombination of key character in tetraploid species of Triticum. Studies on genetic differentiation in tetraploid Triticum species by mutation and hybridization experiment revealed that the key character fall into two distinct groups, one of core combination and mutation and the other of peripheral character, ear density, beak length and glume shape, which recombine and mutate freely the importance and relative contribution of such characters in evolution. Pokrovshii (1972) has reported some results of breeding durum wheat. As a result of breeding work at the Institute of Unirrigated Agriculture in Uzebekistan, three varieties have been bread of which one Altyn, has been approved in the republic and two others Dzhura and Len curum 3 are undergoing state varietal trials. Among the most promising new variety are melanopus 1, Melanopus 2 and Melanopus 110. Katarina Borojevic and Borogevic have studied the response of different genotypes of Triticum aestivum sp. vulgare to mutagenic treatments of quantitative characters in the M. generation. The mean value increase sharply in M_2 composed with M_1 but are still below

those of control. In M_3 a slight decrease was observed, in the M_4 a stagnation and in M the means value are around those of control in all genotypes.

In the individual genotypes are considered in which the means has reached or exceeded the means of the control in the M_4 and M_5. It was very difficult to see the response of different genotypes. In general, all genotypes react in the same way. The specific response to mutagenic treatments was not found in the studies of the quantitative character of the seven high selected varieties of Triticum aestivum vulgare in later generations. Vyas (1974) has conducted intraspecific hybridization for the synthesis of winter durum wheat. The results are given of a study of F_1 to F_3 hybrids. The characteristics of the F_1 are presented in relation to germination and survival rates. Winter hardiness, inheritance of the morphological characters of the ear and heterosis. During segregation in the F_2 new forms appeared in addition to those resembling the parental varieties. In the F_3 stable homozygous forms with useful economic and biological characters were obtained and are now being studied. Naskidashvili (1974) has reported results of study of interspecific hybrids in the second generation. The results are presented of study of hybrids obtained from crosses of varieties of bread wheat with nonbranching forms of Triticum turgidum. The number of different forms which segregated in the F_2 varied between 12 and 25 with direct

crosses and between 10 and 20 with reciprocal crosses, according to combination useful initial materials for breeding. Kovarskii, Georgiev and Buyukli (1974) have reported results of a study of interspecific hybrids obtained by crossing winter durum and bread wheat. A study was made of F_1 hybrid for seed set, germination and survival rates and winter hardiness. In phenotype the F_1 intermediate between their parents. The highest heterosis observed was for height (17%) and number of spikelet on the main ear (16%). The fertility of the F_1 hybrids was 40 to 50%. The F_2 hybrid segregated into parental and intermediate forms. The former were twice as fertile as the latter. In the F_3 further segregation occurred for morphological and biological characters. Useful transgressive forms were obtained including some yielding 30.6-38.8 c/ha. Sheredeko and Kartushuyak (1975) have studied inbreeding and heterosis in wheat. The inbreeding of plants with chasmogamom flowers led to some depression in the form of reduced height and seed yield and a lengthened growth period. Useful lines were obtained with short straw, a high protein content and good backing quality. Hybrids obtained by crossing these showed greater heterosis for yield then intervarietal hybrids then yield was 2 to 3 times higher than that of micronovka 808. Kyzlasov (1975) has reported the occurrence of new genotypic formations after the hybridization of winter with spring wheats. When seven winter forms were crossed with seven spring ones,

hybrids were obtained which differed from their parents in straw length. Caryopsis shape, length of the period emergence headling and also in the manifestation of hybrid necrosis, nacrosis was lethal in crosses of Timiryazevskaya with Narodnaya Kharkov 46 and Raketa. In PPG 1 x Skala, necrosis did not occur in the F_1 lent necrotic forms segregated in the F_2. In the F_2 Rannyaya 12 x skala, the segregation ratio was 9 necrotic; 7 normal when PPG 1 was crossed with saratov 36 and lutexens, 62, dwary forms segregated in the F_2 in the ratio 8 normal, 1 dwary. In some other crosses, dwary forms segregated in the F_2 and later generation. The selection of plants with shortened and lenthed caryopses in the hybrids Bezostaya x saratov 29 was found to effect 1000 grams weight a longer caryopsis 1 gave a higher 1000 grams weight.

Mettin (1975) has worked on testing the genetic effect of donor chromosomes in intraspecific substitution in wheat.

A combine F_1/F_2 analysis was used in which 34 monosomic and disomic hybrids from crosses of monotelosomics 4A and 5A of poros with ten winter and two spring wheats were studied in the F_1 and (2) four 42 - chromosomes populations with partial or complete recombination. From the crosses with gains and Atlas 66, were studied in the F_2. The F_1 analysis showed that chromosomes 4A was critical for single plant yield in six out of ten combinations but that it was not critical for plant height, number of ears/plant, heading date or ear length,

chromosomes 5A proved critical for number of ears/plant in four out of seven combinations but only in some cases for the other traits studies. The F_2 analysis, together with the frequency distributions of the populations, provided evidence that minor factors for plant height, number of ears/plant, ear/length, number of grains/spikelet. Single plant yield and protein content of the grain are associated with chromosomes 4A in the two crosses studied.

Larik (1975) has investigated induced mutations in quantitative character of Triticum aestivum. Increasing the dose of gamma radiation on seeds of four varieties resulted in increased variance, heritability estimate and expected genetic advance for plant height, culm diameter and days to heading. The means of these character decreased compared with untreated controls. Mean 1000 grain weight increased. After irradiation, Wisconsin Supremo had the highest hereitability estimates and genetic advance for plant height and the greatest reduction in height the variety No. 43 had greatest reduction in days to heading and Wisconsin supremo had the highest genotypic coefficient of variation and the highest estimate of heritability and genetic advance.

Chapter 9.

9. BREEDING OF MAIZE

As example the work on maize has been described.

Maize is the leading cereal crop in the America. In the United States it occupies nearly onefourth of the total cropland and has a value double that of wheat, the second most important crop. In India maize occupies fifth place in acreage and fourth place in production among the cereals grown. Maize is grown on limited acreage in other countries of South and Southeast Asia. Maize is a relatively recent introduction to this area of the world. Apparently being brought to Southeast Asia from America by Portugese.

9.1. Pollination in maize

An understanding of the methods of breeding maize is dependent upon a knowledge of its pollination methods and upon the genetic composition of the maize plant. Maize bears monoecious flowers with staminate flowers produced in the tassel and pistillate flowers on the shoot. Pollination is accomplished by the transfer of pollen from the tassel to the silks. About 95% of the ovules on a shoot are cross pollinated and about 5% are self-pollinated. The main stem of the maize plant terminates in a tassel bearing two-flowered staminate spikelets each flower having three stamins. As tassel flowers open the anthers are pushed out by the elongating filaments and pollen grains are

emptied. A single tassel from a normal plant may produce as many as 25,000,000 pollen grains or an average of over 25,000 pollen grains for each kernel on an ear with 800 to 1000 kernels.

Pollen shedding begins one to three days before the silks have emerged from the husks of the same plant, and usually continues for a period of several days after the silks are ready to be pollinated. Hot dry weather tends to hasten the pollen shedding.

The ear shoots arise as branches from nodes about midway of the stalk. Each shoot is composed of a shank from which the husks arise, and terminates in the ear on which the pistillate flowers are born. The spikelets are born in pairs and since each spikelet normally produced one fertile ovule, there is an even number of rows of kernels but does not normally develop. Fertilization of the second ovule produced crowded and irregular kernels on the ear. Fresh silks function both as the stigma and the style and are receptive to fresh pollen throughout their entire length. Severe drought may delay the emergence of the shoots. Fertilization of the ovule usually occurs within 12 to 28 hours after the silk has emerged. Maize propagated from seed that has been produced by uncontrolled pollination is commonly referred to as open pollinated maize.

9.2. Method used in breeding of maize

Varieties in open pollinated maize have been developed largely by mass selection.

9.2.1. Selection and variety hybridization were tried but were never widely used. Since the development of hybrid maize most of the efforts have been directed towards this method of breeding. In India and some other areas being developed either for use as varieties or as source of germplasm for further breeding.

Breeding open-pollinated maize

Mass selection is the principal method of breeding open-pollinated maize. Most of the important varieties of open-pollinated maize in the Americas originated by this method of breeding.

Mass selection

In the mass selection of breeding maize ears are chosen on the basis of plant and ear characteristics. The ear is the unit of selection because of the necessity and convenience in handling it. Mass selection is used both as a method for maintaining existing varieties of open-pollinated maize and for developing new varieties. Each cultivator who selects ears of maize to plant his next years crop becomes a breeder, and he can change the character of the maize he grows by selecting for a specific type and characteristics mass selection was not generally considered effective in earlier years for increasing the yield of an adapted variety. The ineffectiveness of mass selection for increasing yield result from (a) the breeders inability,

due in part to poor experimental techniques, to recognize whether a particular plant was superior due to its genotype or to the specific environment in which it was growing (b) superior plants being pollinated from both superior and inferior plants so that the high yield potential of a plant not reduced in all of its offsprings and (c) the fact that rigorous selection for specific plant characteristics often led to inbreeding and thus actually decrease yields. There was an indication that in open pollinated maize most progress may be made by selection for (a) vigorous, strong plants (b) large sound, well developed ears, (c) earns from disease free plants and (d) proper maturity.

Variety hybridization

Hybridization between varieties either intentional or accidental, was responsible for the origin of many commercial varieties of open-pollinated maize. Such hybridization added to genetic variability and often new varietal types could be evolved.

In 1880, Dr. Beal, at the Michigan Agricultural Experiment Station in the U.S.A., described an experiment in variety hybridization in which one variety grown in adjacent to row. An increase in yield was obtained in the hybrid progeny. A plan by which farmers could produce their own crossed seed was later outlined.

9.2.2. Hybrid maize

Early attempts to improve the yields of open-pollinated

maize were mostly disappointing although varieties adapted to various production areas had been developed. While it was possible to develop many different varieties or to change the characteristic appearance of a variety raising the inherent yielding ability of a well established variety. This failure to improve the yield arose from the heterozygous nature of open pollinated maize and the poor plot techniques used at that time. The inherently high yielding plants are the result of favourable gene combination. They are not always reproduced in the progenies of the high yielding plants since the plants are fertilized by pollen from plant which are highly heterozygous.

Hybrid maize is the first generation progeny from a cross involving inbred lines. The breeding of hybrid maize involves (a) development of inbred lines by controlled self-pollination, (b) determination of which lines may be combined into productive crosses and (c) commercial utilization of the cross for seed production.

Inbred lines

An inbred line is a pure line developed by self-pollination and selection until apparently homozygous plants are obtained. This usually requires five to seven generation on inbreeding. Since maize is normally cross-pollinated, pollination must be controlled in each generation and the silks must be pollinated

by hand with pollen collected from the tassels. After an inbred line is developed it may be maintained by self-pollination.

Inbred lines were developed originally from open pollinated varieties. If a maize plant from an open pollinated variety is selfed, the progeny will be reduced in vigour as compared with the parent plant. Additional reduction in vigour may be noted with each selfed generation until a homozygous or true breeding line is developed. About one half of the total reduction in vigour comes in the first generation of selfing, the remaining loss being halved with each successive generation so that losses being halved with each generation and are small after three to five generations. In addition to loss of vigour, individual plant in the early selfed progenies exhibit many faults such as reduction in plant height, tendency to suckering, lodging, disease susceptibility and a wide assortment of other undesirable characteristic. The desirable plants are selected for selfing again in each generation, and the weak abnormal plants are discarded. After five to seven generation of inbreeding and rigorous selection, vigorous inbred lines, uniform in appearance, are developed. Each inbred will have a different combination of genes. An inbred is a line and is discended by self pollination from an apparently true breeding plant. Hence, each plant will look exactly like every other plant with the same inbred line. The purpose of inbreeding is to fix desirable character in a homozygous condition in order that the line may

be maintained without genetic change. Vigour exceeding that lost during the period of inbreeding is regained in the F_1 progeny when the inbred is crossed with related inbred. During the inbreeding process many undesirable recessive genes that reduced yield, which are musked by their dominant allele in an open-pollinated variety, are eliminated as the weak and undesirable plants are discarded. The desirable characteristic of the inbreds, such as strong stalks and disease resistance, are transmitted to the hybrid progenies when the inbreds are crossed.

Here are a few selected examples of work done on maize. Beadle (1933) has conducted studies in asynaptic maize. Synapsis occurs, apparently normally in asynaptic maize plants.

Homologous chromosomes tend to separate during pachytene without chiasma formation. Chiasma frequency is low at diplotene, diakinesis and metaphase. The degree of metaphase pairing in asynaptic plants is variable. The plant studies showed the maximum possible range in model frequency of number of bivalents, namely from 0 to 10. There is a positive correlation between number of bivalents at metaphase and minimum number of chiasmata which must be assumed to account for the observed associations. The asynaptic gene is located in the P - br chromosome approximately midway between the P and br genes.

Parthasarthy (1953) studied the use of induced autopolyploids in plant breeding. Induced autotetraploidy can, therefore, be

a useful line of work for the plant breeder if only studies are undertaken on the processes following polyploidy rather than the polyploidy induction itself.

There is no doubt, therefore, that new autopolyploid types as in agriculture, if only the right genetic combinations and proper agricultural environment are found. The methods of determining these combinations and proper agricultural environment are found. The methods of determing these combinations and of selecting for vigour and fertility must be considered as major problems for further investigations in the field of autopolyploidy.

Prasad, Joshi and Swaminathan (1972) studied mutation and recombination of key character in tetraploid species of Triticum.

Studies on genetic differentiation in tetraploid Triticum species by mutation and hybridization experiments revealed that the key character fall into two distinct groups. One of core character, threshing habit, which showed rare recombination and mutation and the other of peripheral character, ear density, beaklength and glume shape, which recombine and mutate freely. The important and relative contributions of such characters in evolutions.

Ahlcowahia and Dhawan (1972) studied asynapsis in maize from Sikkim. Asynaptic plants with upto 20 univalents were found in a maize variety from Sikkim. This maize population is an

isolated and distinct cultigen with characters of primitive maize races.

Annenkova (1973) studied the effect of the pollen parent on heterosism in maize.

The results are presented of a study of the effect of the male parent on yield, earing and ripening rate in double interline hybrids. The choice of a suitable pollen parent enabled the production of heterotic hybrids exceeding the standard in yield by 21.8-41.2% and ripening rate by 41.7-47.0%.

Lorenzoni (1973) studied the present trends of maize breeding. Aspects of maize breeding to which the attention is particularly drawn include hybrids or to breed synthetic varieties, the problems associated with cytoplasmic male sterility. (The susceptibility of cytoplasm T to race T of cochliobolus heterostrophus and the high incidence of fertile plant with cytoplasm) The use of the gene Ht for resistance to C. heterostrophus and the use of gene ae (amylose extender) Wx (waxy) o_2 fl_2 (Opaque - 2 & floury 2) to improve the nutritional quality of the grain especially the protein and lysine content.

Nigam, Paliwal and Asnani (1973) have conducted genetic analysis of stalk strength in maize. Genetic analysis for rind thickness, crushing strength and stalks breakage indicated that both additive and non-additive genetic variances were important in the determination of these characters. However, in case of

crushing strength and stalks breakage additive, genetic variance played greater role. Dominant gene were responsible for higher crushing strength and rind thickness and for lower stalk breakage. Average degree of dominance was in the range of over dominance for crushing strength and rind thickness and for stalk breakage it was in partial dominance range.

Mahboob Ali and Ravindranath (1974) studied performance per se in cross combinations of ten inbred lines of maize collected from four locations in India. Significant and appreciable differences were observed within maize inbred line from various colour, anther colour, silba colour, tassel type, grain type, grain colour and cob colour. The shift in flowering period have made production of some of the specific single crosses and hybrids difficult. It is suggested that selection by various breeders in material not completely homozygous was responsible for such changes. The lines differ in their combining ability as well. The same hybrid built up with inbred lines maintained at one location gave significantly different performance than when inbred lines from other locations were used. Parental inbred lines should, therefore, be released only after they have attained complete homozygosity. They should be maintained by alternate selfing and sibbing within the regions for which the hybrid is released.

Kazanko and Panomarenko (1976) have described the results of study of two-earned single hybrid and their parental forms.

A comparison was made of two eared maize hybrids and their initial lines with a one yeared hybrid and its parental line in density of stand. In the two eared hybrid an increase in density led to a fall in the number of plants per hectere only 50% of plants had two ears and at 60,000 only 10% in one hybrid and 20% in the other. However, at higher densities the yield of the two eared hybrids fell less markedly than that of the one eared forms. Unlike the hybrids, the one eared and two eared lines all gave higher yields at the highest density.

Sokolov, B.P. and Ivakhuenko, A.N. 1976 studied breeding early hybrids for northern area of maize breeding.

A review is presented of recent work at the all union maize institute on breeding early hybrids with good cold resistance mention is made of the hybrids bred. Joginder Singh, B.K. Mukherjee, N.N. Singh, R.D. Singh, N.P. Gupta, S.B. Singh, Iqbal Singh, [illegible], A.L. Lodha and H.O. Gupta have reported that systematic improvement of maize crop was taken up in 1957 under the coordinated maize breeding scheme at this division. In a period of less than four years, first four maize hybrids, namely Ganga 1, Ganga 101, Ranjit and Deccan were released for general cultivation in 1961. A number of more productive widely adapted and disease resistance hybrids namely, Ganga safed 2, Hi Starch, Ganga 3, Ganga and Ganga 5 were subsequently released and are already popular.

To overcome the problem of recurring hybrid seed production, a number of high yielding composite have also been released. Composites like Kisan sona, Vikram and Jawahar developed in recent years at this division have not only given high yields, but have also known considerable to major disease and pest.

Development of high yielding hybrids and composite varieties

A number of high combining inbred lines were selected from the four reciprocal recurrent programmes initiated in 1964 at various centres of the coordinated maize improvement scheme. The selected lines were used in synthesizing the improve double cross-hybrids which are under test in all over India.

Vencovsky, Paterniani, Miranda Filho and Ando have studied the effect of gamma irradiation on a maize variety. While the average grain yield of progeny grown from irradiated seeds of centramex was unaltered the additive genetic variation was increased in separate years. If the two years of trails were analysed jointly heritability coefficient for progeny over aged 44.6% and 7.5% and selection progress between progenies was 6.5% and 1.1% for control and irradiated material respectively.

Khristova has conducted genetic analysis of some quantitative characters determining yield in maize.

In the study of ear length and number of rows per ear using diallel crosses involving eleven lines ear length was controlled by dominant genes of which the lines S 103 had the

largest number and number of K rows showed, in complete dominance or over dominance. The lines S 103 S 261 - 1 and Sh 144 had the highest general and specific combing ability for ear length and the lines 142 S 177 and S 261-1 for number of rows per ear.

Chapter 10.

10. BREEDING OF TOBACCO

As example the work on tobacco has been described

Although native to the Americas, tobacco is now grown extensively in all of the countries in South and Southeast Asia. Tobacco was introduced into India during the early part of the 17th century and is presently grown on an area of about 4 lakh hectares. India is third among all countries in production of tobacco. The kind and quality of tobacco grown is greatly influenced by the soil and the climate.

10.1. Methods of breeding tobacco

The principal methods of breeding tobacco are introduction, selection and hybridization. Modern breeding work on tobacco was started about the beginning of the present century in the U.S.A. and other countries. Through the efforts of the Dutch scientists breeding work on tobacco was initiated in Indonesia which led to the development of some excellent strains of cigar wrapper tobacco for that area. Breeding of tobacco has been in progress in the Philippines for many years.

Unlike crops grown for their seeds, the economic value of tobacco lies in the quantity and quality of the leaf.

10.1.1. Introduction

Introduction has played an important role in the establishment of tobacco varieties in India. Tobacco was first introduced

in India by the Portuguese in the beginning of the 17th century.

10.1.2. Selection

Pure line selection has been the main method of breeding in the establishment of most of the improved tobacco varieties in India. Selections have been made from some introduced varieties such as Harrison special 9 from Harrison special and chatham from unselected cross made in chatham, Virginia U.S.A.

The local tobacco grown in India has a wide range of variability. The Lanka varieties grown for many years along the Krishna and Godavari, have been found to be quite variable genetically.

Hybridization

As in other crops, hybridization became more important in breeding tobacco as plant character could be obtained more or less to fit the breeder's design. Large number of improved varieties have been developed by hybridization, principally in the U.S.A. in which were combined genes for larger number of leaves improved qualities and disease resistance.

10.1.3. Interspecific hybridization

Interspecific hybridization has been an important procedure in the breeding for disease resistance in the U.S.A. In many instances resistance genes for specific disease could be

found only in other species of Nicotiana. This necessitated the use of interspecific crosses as already described with interspecific crosses, deleterious genes are often added to the adapted variety along with the genes for disease resistance. To overcome these undesirable features and to recover the plant type and quality characteristics of the adapted variety type, back crossing has been used also with intervals crosses, but the intensity of back crossing is not generally as great as with interspecific crosses. The large number of species of Nicotiana which have resistance to common destructive disease to tobacum makes this a rich field for the breeder.

10.1.4. Mutation breeding

Use of irradiation or chemical mutagens for creating variability has already been demonstrated. Although creation of a large number of viable mutations is possible in tobacco due to its amphidiploid genotype, the quality requirements of the plant makes it difficult to obtain a desirable mutant directly. Hybridization may be necessary to transfer the desirable mutant character to adapted varieties. Progenies of each capsule should be advanced separately following irradiation.

10.1.5. Polyploidy

Since cultivated tobacco is already a polyploid plant, achieving success by production of polyploids in tobacco may be difficult. Polyploidy is useful in making interspecific crosses

for the transfer of disease resistance or other desirable characters.

Some selected examples of work on tobacco are described below:

Smith (1939) studied the induction of polyploidy in Nicotiana species and species hybrids.

Meristem treatments with 0.4% colchicine and seed treatment with 0.2, 0.4 and 0.8% colchicine were made on a number of Nicotiana species and species hybrids, polyploid forms of the following were obtained:

Type	Treatment	No. of polyploid
N. glauca	Seed	4
	Meristem	1
N. rustica	Meristem	1
	Seed	4
N. tobacum	Seed	1
	Meristem	1

Seed and polyploid offsprings have been obtained from N. rustica, N. tobacum rustica x tobacum hybrids x glauca and tobacum x sylvestris.

Dawson (1942) studied nicotine synthesis in excised tobacco roots. The ability of excised tobacco root tips to synthesize nicotine when grown in sterile culture has been investigated.

In contrast to all other organs of the tobacco plant the roots were found to manufacture nicotine in appreciable amounts

as growth occurred.

Smith (1943) studied induced heteroploids of Nicotiana. Among plants of Nicotiana Langs dorffii (2n = 18) affected permanently by colchicine treatment 25% were off type. These were found to be heteroploids with somatic chromosomes numbers as follows: One 9 (a haploid) one had 17 (2n = 1) two had 32, two had 33, four had 34, two had 35 (4n - 1) and one had 72 - chromosomes. L. (an actoploid).

Clayton (1953) studied control of tobacco disease through resistance. Resistance to the major tobacco disease can be found in N. tobacum but so far it has been possible to utilize less than half of this resistance in commercial tobacco varieties.

Immunity or very high resistance to the major tobacco disease has been found in various species of Nicotiana. All indications are that these transfers from distantly related species, once established in the tobacco genomes can be used for more rapidly and with fewer complications involving type yield, and quality, than is the case with any resistance found within the cultivated species.

Murty, Murty and R. Anantharaman (1962) conducted studies on indigenous tobaccos and the improgement of Lanka tobacco.

Here the superior performance of an improved variety of Lanka tobacco which has been designated Dr. I. An improved method of harvesting Lanka tobacco has been suggested on the

basis of the experimental results. A method of grinding Lanka tobacco has also been suggested.

Burk and Heggested (1966) studied the genus Nicotiana as a source of resistance to disease of cultivated tobacco. Nicotiana tabacum L. cultivated tobacco, is one of 65 species in the genes Nicotiana over the past 30 years, varying numbers of the species have been included in test of resistance to tobacco disease by research workers in tobacco producing areas throughout the world. This report summerises these investigations in tabular form and discussed method whereby interspecific transfer of disease resistance may be accomplished.

Chapter 11.

11. INDUCTION OF POLYPLOIDY

11.1. Introduction

Polyploidy means doubling the chromosome number and cells containing more than two haploid sets of chromosomes from triploids on up are polyploids. There are two major types according to the origin of the chromosomes. Polyploids having chromosomes derived from parents with similar genomes are autopolyploids, whereas having chromosomes derived from parent with different genomes are allopolyploids, e.g., if the chromosome of one parent are designated as A A and the chromosome of the other parent as A A the pairing of the chromosome in the resulting autopolyploid is consistent with their similar genetic constitution. On the other hand, if the chromosome of the second parent are designated as B B, the pairing in indicative of significant differences in genetic make up. An autotetraploids chromosomal content may be designated as A A A, an autotetraploids as A A A A, and an allotetraploids as A A B B, where each letter represent a haploid set of chromosome of specific genetic composition. A triploid organism may arise from the union of a tetraploid individual and a diploid individual. An autotetraploid may arise naturally from a doubling of the diploid number in somatic cells or from the union of two diploid gametes.

The phenomenon of polyploidy or the existence in genetically related types of chromosome number which are multiple of each other is one of the most widespread and distinctive feature of the higher plant and was one of the earliest of their cytogenetic characteristic to become extensively studied.

Many of our most valuable crop plants such as wheat, oat, cotton, tobacco, potato, banana, coffee and sugarcane are polyploids was supplemented by decisive evidence concerning the actual percentage of some of them as in the example of Triticum aestivum, Gossypium hirsutum and Nicotiana tobacum. The role of polyploidy in the origin of new types being created by the plant breeder was recognized in the case of "perennial wheats". An increase in size of the individual cell is perhaps the most widespread effect of polyploidy.

A number of secondary effects are associated with the primary effect of polyploidy on cell size. Effect of polyploidy is on the genetics of segregation due to the presence of duplicated genes.

The polyploidy is the best way of producing constant and fertile species from sterile interspecific hybrids, e.g., Nicotiana digluta. Typical allopolyploids or amphiploids which resemble diploids in the regularity of chromosome pairing at meiosis and in the constancy of their genetic behaviour have now been synthesized in forty or more instance. At least half of the naturally occurring polyploids are probably strict

allopolyploids in that they have originated from F_1 hybrids between two ancestral species which were so distantly related to each other that their chromosome were essentially non-homologus. This does not include species which are diploid with respect to the basic number of their genus, but of which this generic number may be of polyploid derivation, as in the subfamily Pomoideae of the Rosaceae.

There are now several examples which show that even extreme allopolyploids, derived from hybridization between species having widely different chromosomes may resemble one or the other of their parental species so closely that they have not been recognized as distinct by systematists. One of the most striking of these is Nasturtium micronhyllum. This species was originally regarded by Manton (1935) as an autotetraploid form of the ordinary water cress N. officinales chiefly on the grounds of its external morphology.

11.2. Types of polyploids and their characteristics

The four types of polyploids recognized by the writer (1947) are:

Autopolyploids, segmental allopolyploids, true or genomic allopolyploids and autoallopolyploids. The first two occur in nature predominantly or entirely at the level of triploidy or tetraploidy; true allopolyploidy can occur at any level form tetraploidy upwards while autoallopolyploidy is confirmed to hexaploidy and higher levels of polyploidy. The term amphiploid

coined by Clausen, Keck and Hiesey (1945) is suggested as a collective term to cover all types of polyploids which have arisen after hybridization between two or more diploid species separated by barriers of hybrid sterility. It, therefore, includes segmental allopolyploids, true allopolyploids, and autoallopolyploids plus aneuploids which have arisen from hybridization between two species belonging to an aneuploid series with lower numbers as in Brassica Nagahuru U, (1935) Frandsen (1943) and Erophila (Winge 1940). The second type of polyploid is that termed by Stebbins (1947) a segmental allopolyploid. It may be defined as a polyploid containing two pairs of genomes which possess in common a considerable number of homologous chromosomal segment or even whole chromosomes, but differ from each other in respect to a sufficiently large numberof genes or chromosomes segments.

Another well-known polyploid which probably is of this type is Solanum tuberosum. Its somatic number is 2n = 48, which makes it tetraploid. Since the basic number for Solanum as well as a large number of other genera in the Solanaceae, is x = 12.

Two other well-known polyploids which are probably of this type are Phleum pratense and Solanum nigrum. The former species was judged by Gregor and Sansome (1930) to be an allopolyploid, an opinion shared by Clausen, Keck and Hiesey (1945). But Nordenskiold (1941, 1945) after intensive study, considered it to be an autopolyploid containing the diploid complement of Phleum nodosum trebled, while Myers (1944) noted cytological

characteristics indicating autopolyploidy. But since two different haploid plants of P. pratense, one studied by Nordenskiold and one by Levan (1941), typically form 7 bivalents and 7 univalent their genomic formulae must be AAB, and that of the diploid AAAA BB. The evidence of Nordenskiold suggests that the A genome is very likely that of P. nodosum and the B genome, that of the diploid P. alpinum. The tetraploid P. alpinum, which Gregor and Sansome believed to be ancestral to P. nodosum was shown by Nordinskiold (1945) to be an allopolyploid containing the B genome and a still different one, belonging to some diploid species as yet un-identified. The naturally occurring polyploid relationship found in the Brassica species is three common diploid species of Brassica, B. campestris, B. nigra, and B. oleracea, have haploid chromosome number of 10, 8 and 9 respectively. These have been assigned the genomes designations A, B and C. B. juncea (AABB) is a natural amphidiploid combining the genomes of the two species. B. campestris (AA) and B. nigra (BB). B. napus (AACC) is a natural amphidiploid combining the genomes of the two species, B. campestris (AA) and B. oleracea (CC). B. carinata (BBCC) is a natural amphidiploid combining the genomes of the two species, B. nigra (BB) and B. oleracea (CC).

Polyploidy is of special interest in plant breeding because it adds to the genetic diversity in the plant kingdom. Polyploidy offers the breeder an opportunity to bring about changes

in the character of a plant by altering the chromosome number and consequently the number of genes within a single cell. Another consequence of polyploidy is to increase the complexity of genetic ratio. In polyploid species genes frequently occur in multiples of the basic chromosome number. In common wheat a hexaploid which originated from a combination of the chromosome from three different species have been reported and to be determined by three independent genes. In a polyploid species recessive plants appear much less frequently in a population than they would in diploid species. This requires that the breeder grow a much larger population of a polyploid to recover a corresponding number of recessive phenotypes than would be necessary with an ordinary diploid. On the other hand, recessive mutations that are deleterious to the parent may be covered up by their dominant alleles to a greater extent in polyploids so that they are not expressed as frequently in the phenotype of the plant.

Polyploidy has been an important factor in the evolution of plant species. A knowledge of its cross from closely related species, chromosome doubling is frequently necessary either in one of the parent species before the cross is made or in the hybrid plant to obtain fertile progenies from interspecific crosses.

11.3. Brief account of earlier work on polyploidy.

A few examples of work on induction of polyploidy are given below:

Wellensick (1938) studied methods for producing Triticales.

1. F_1 plants of crosses between winter wheat and winter rye were vegetatively reproduced by sowing in the spring; followed by repeated division clones of 300-400 plants could be obtained in one season.

2. F_1 clonal plants were treated with colchicine by putting them with their roots in an aqueous solution. The most promising result were obtained with 0.05% solution during a four day period in day time in the solution over night in water.

3. Several new Triticales with 50 chromosomes have been produced

Nebel and Ruttle (1938) studied the cytological and genetical significance of colchicine.

Colchicine inhibits spindle formation. In dividing cells of animals and higher plants, cells with the doubled chromosome number are thus formed. As growth progress in plant, didiploid and mixochemeric shoots are formed. Tetraploids have obtained by this means in many do not show the effect of polyploidy equally well. To induce chromosomal changes in somatic tissues of cultivated plants as obtained with colchicine may be called plant breeding with non Mendelian methods. It must be emphasized that this method of genetical approach will require more care on the part of the practical breeder and more expense than the Mendelian method.

Emsweller and Brierley (1940) studied colchicine induced tetraploidy in Lilium. As a result of colchicine treatments

of the stem apex of Lilium formosanum, bulblets were produced, most of which proved to be tetraploids. The tetraploids produced on one individual plant were all slightly fertile but only one of nine produced on another individuals was fertile. The tetraploid flowers varied in size but average about 25% larger than diploid flowers. Six hundred and six seedlings were secured from selfing the colchicine induced tetraploid and seven of the original 22 tetraploid have been by scaling.

Mendes (1940) studied polyploid cottons obtained through use of colchicine.

Gossypium hirsutum.

Octoploid and tetraploid plants were obtained from seeds of Gossypium hirsutum and G. herbaceum treated with colchicine. The seeds of G. herbaceum were generally homozygous and reacted as a rule more uniformly to the drug than did the seeds G. hirsutum

Flower with dehiscent anthers were self pollinated and other were cross pollinated with pollen from tetraploid and octoploid plants. No fruit-set occurred. A few fruits from open pollinated flowers have been obtained. There were almost spherical, larger and havier than the normal. The seed coat was very hard and the percentage of germination was low. These seeds yielded normal tetraploids (2n = 52).

Masima (1942) conducted studies on the tetraploid flax induced by colchicine. By treating with 0.08% colchicine solution

in 20 hrs. at 25° C tetraploid plants were induced in 2-5% of the treated seeds in 3 species. These tetraploid plants were of gigas types.

Hecht (1944) induced tetraploidy in a self sterile Oenothera. Tetraploidy has been induced in a self sterile race of Oenothera rhombinetala by treatment with colchicine. An analysis of the inheritance of the self sterile allels in the tetraploids based on opposing sterility allels according to the general scheme of East and Mangeldory.

Parthasarthy (1953) has studied the use of induced autopolyploids in plant breeding.

Induced autopolyploidy can, therefore, be a useful work for the plant breeder if only studies are undertaken on the processes following polyploidy rather than the polyploidy induction itself. There is no doubt therefore, that new autopolyploid types as in agriculture, if only the right genetic combination and proper agricultural environments are found. The method of determining these combinations are found these conditions and of selecting for vigour and fertility must be considered as major problems for further investigations in the field of autopolyploidy.

Simonet, Marc and Peter Werckmeister have conducted a cytogenetic and descriptive study of the trispecific iris hybrids.

Stolorine which has curious colour is interacting for its vigour and its early flowering. If male fertility permits the study of its breeding possibilities as a parents with other tall garden irises.

Mahal, Pal and Khoshoo (1968) have described artificial and natural polyploids in Antirrhinum.

It is pointed out that the cytogenetic differences between the two phytogeographic elements of Antirrhinum. The European species appear to be at diploid level with genomes not sufficiently differentiated while the American species tend to be polyploid. The gene flow and consequent break down in self incompatible barriers. If future cytogenetic studies confirm their differences, then Antirrhinum is a divergence in similar environments in two different continents.

Bose and Panigiohi (1969) have conducted studies on induced polyploidy in Zinnia linearis (Benth).

Apical growing regions of 2 varieties of Zinnia linearies orange and white were treated with 0.2% cochicine for 12 hrs. Survival of plants till maturity was less in colchicine treated ones than in controls. Typical effect of cochicine like slow growth of plants during early stages of the development, thicker, leathery and deformed leaves, shorter internodes, increase basal circumference and more number of leaves were observed in the treated plants in comparison with the control one. In addition to this, colchicine treated ones of both the varieties had less

number and bigger size of stomata in comparison with the control.

Raghuvanshi and Chawhan (1974) have conducted studies on artificially induced higher polyploids of Cartharanthus roseus.

A comparatively account of artificially induced tetra, mixo and octoploids in C. roseus is presented multivalent frequency was very low multiple and multipolar spindle were normally in mixo, hexa and octoploids, while spindle functioned normally at 2n and 4n levels complements fractionation in origin of anomalous spindle.

Zadoo, Roy and Khoshoo (1975) studied cytogenetics of cultivated Bougainvilleas.

Induced tetraploidy and restoration of fertility in sterile cultivars.

Induced tetraploidy restores fertility in sterile cultivars of induced tetraploidy of self incompatible, but set seeds readily on crossing.

Grant (1976) studied the evolution of karyotype and polyploidy in arboreal plants. Although biochemical studies have been carried out on woody plants interesting correlations have been noted between nuclear DNA content and several parameters. Such a geographic range ecological adaptation nuclear volume and karyotypic differences such as chromosomes length. Hybridization between genomes with complements possessing chromosomes of different relative size, B chromosome and repetition DNA and heterochromatin B chromosome may not be as rare in three species were genomes at

present exhibit only moderate differences between species while successful callus production has been induced for a number of woody species. So far no haploid trees have been produced. In Betula there appears to be little barrier to cross-fertilization and plants with different euploid chromosome have been obtained from seed to the same parental tree.

Dermen Haig (1940) conducted a cytological analysis of polyploidy. The methods of inducing polyploidy in the meiotic and somatic cells of Rhoeo discolour with colchicine and temperature. Gross and cytological observations are presented concerning somatic changes brought about by colchicine treatment of Rhoeo flower parts. It was found that colchicine enters tissue and inhibit cell divisions, while the nucleus and cell volume may increase presumably as long as colchicine is present in sufficient quantity to be effective until such an increase is checked by some other factors. In temperature treated materials fragmentation, fusion and chromatin bridging occur. It is suggested that clumping brought about by temperature treatment is responsible for these features.

Chapter 12.

12. INDUCTION OF MUTATION

12.1. Introduction

Mutations are sudden heritable changes in an organism whereby its progeny may exhibit altered size, form and function. They are classified into those involving changes in the chemical structure of gene (point mutation), those involving chromosomal aberrations (Translocation, deletion, inversion, duplication). And those involving changes in chromosome number.

The mutations occurring constantly in nature or in experimental culture are called Spontaneous mutations. It is not a promising source of immediate variability to the plant breeders. However, mutation can be artificially induced and induction of vital beneficial mutation is now becoming an increasingly important method of plant breeding.

Regarding the history of treatment of embryos and seeds with mutagens. Muller (1927) was perhaps the first person to explain the effect of X-rays as biological organism and after this Sach Wery, Stadler, Riby studied the effect of various mutagens in different crop plants. Later Katarina and Borojevic gave mutagenic treatment to several varieties of Triticum aestivum spp. vulgare in order to study the response of different genotypes. C. Broerljes worked on Saintpaulia to study the dose rate effects of various mutagenic agent both radiation and

chemical mutation frequency and spectrum Å Gustaffason U. Lundginst and G. Ekman treated grains for 1, 2, 3, years (1960-62) either singly with neutrons or E.M.S. or with both i.e. mutation research was done on Barley. Roses and Marigold by H. Heslat. More than 1000 mutations of rice have been induced in a single variety by radiation treatment and their agronomic characteristics have been studied by T. Kawai.

Wettsien (1941) has described mutation in Antirrhinum maius which produces equally drastic changes in the flower but which is the less fertile and viable. Pulima A. Desai has treated three varieties of Triticum durum with fast neutrons high doses of gamma rays and E.M.S. solution.

12.2. Importance of mutation in evolution and in plant breeding

The importance of mutation in the evolution of plant species has long been known. But it is only in recent years that means have been available for the practical plant breeder to create at will and utilize mutations for the development of improved agricultural varieties. The knowledge that radiation and chemical mutagens will increase the mutation rate in a crop species has led to the development of a new breeding procedure sometimes referred to as Mutation Breeding. As a tool of the plant breeder, mutation breeding is yet in the developmental stage. The discovery that polyploidy can be artificially induced by the use of colchicine and other means has stimulated the practical breeder to utilize variants created by doubling

chromosome number or by combining chromosome sets from species hybrids as source of new breeding materials.

Mutation occupies a central position in genetics. The mutated gene is the unit of Mendelian inheritance. It underlies those processes which often by exceedingly intricate paths lead to the appearance of genetically determined differences between individuals. It is the ultimate source of hereditary variability, without which evolution would have been impossible. Most branches of genetics deal with mutated genes, but they treat them as existing objects of research without inquiring into their origin and nature. Mutation research on the contrary aims at elucidation the manner in which mutations arise and the way in which the mutated genome differs from the original one.

The first to use the term mutation in a genetical sense was De Vries one of the three rediscoverers of Mendel's laws in 1900. He applied the term to sudden hereditary change which he had observed in the evening primrose Oenothera lamarkiana. As it happen we know now that most of these changes were not mutation in any sense of our modern term, but rare recombinations of existing genes occurring in a peculiar genetical system. The term mutation has been retained to define sudden changes of genotype, excluding the trivial ones which are due to recombination at meiosis or during somatic crossing over.

12.3. Causes of mutation

Gene mutations have been artificially induced by several

agents but most important of them are of two types (a) Radiations of various type (b) Mutagenic chemicals.

These agents are increasingly used in plant breeding for producing variability to the extent desired effect through modifying the gene controlling systems. These have been used in maize and other crop plants extensively.

12.3.1. Radiation

Mutation inducing radiation are of two kinds, ionizing and nonionizing. Ionizing radiation is very important for plant vigour. Beta and gamma radiation X-rays and neutrons are a few examples. The only genetically active nonionizing radiation is ultraviolet light obtained from mercury light.

12.3.2. Ionizing radiation

The effect of radiation chiefly x-rays, gamma rays greatly increases the mutation frequency of genes under certain conditions upto 1000 times that of controlled (Standler on Barley, maize, Good spead on Nicotiana) Nilson Ehle (1929) initiated experiment to induced mutation in cereals and other crops. Gustoffson (1930) used in Barle y.

Generally atoms are neutral and balanced due to positive particles of nucleus and negative particles surrounding nucleus but when ionizing radiation pass through matter then electrical disturbances occur due to addition or loss of electron etc.

The particles become changed and they are called ions. When the ions are formed then some chemical changes take place and the changed molecule is either a gene or part of a gene. When this modified gene duplicate mutation is produced.

If radiation involved sub atomic particles, they are called particulate type of radiation. If the whole molecules like DNA effected or the chromatids break up these are called non-particulate type of radiation.

12.3.3. Types of ionizing radiation

Plant breeders have induced beneficial mutations chiefly by x-rays, neutrons and radio isotopes.

X-rays

It is popular as source of radiation in plant breeding because its machine is generally available easily to operate and its doses can easy to measured and machine can switch on and off at will. The problem of contamination of residual activity is not existance. Thus doses of x-rays required to cause mutation vary with organisms. Nature of material being irradiated and many other factor, e.g., dry seeds, at high doses, germinating seedling or vegetative parts. The object to be used a doses high enough to cause as many mutation as possible without too seriously damaging the germination grows and the fertility of the plants arising from seeds. Different varieties and species differ in x-rays.

Neutrons

It is being increasingly used with the availability of atomic reacters, fast neutrons can be moderated by graphyte or heavy water in a nuclear reactors until this velocity is reduced to that of thermal neutron. These doses calculation is a bit difficult, here the common unit is (Roentgent equivalent = Rq)

The biological materials exposed to thermal neutrons are usually made mildly active for a short period and must be monetered. Neutron irradiation produces effect differ from those x-rays because here the effect on seeds are uniform. There are higher frequencies of chromosomal mutation and aberration.

Radio isotopes.

Radio isotopes of elements like phosphorous P^{32} and sulphur S^{35} which are essential in nuclear metabolism may be used in a number of ways on mutagenic agents, since the energy in the form of β^- particles it is desipated fairly rapidly. But then enough precautions taken in utilization and disposal. Radioisotopes of phosphorous and sulphur must be used in such a way that they are moved through transpiration streams actively dividing, meristems here they move on ions but being unstable. They transmulate and B-particles causes the chromosomal rearrangement which result in mutation. It has been found in Triticum agropyron hybrid seedlings if treated in petridishes with 40-60 Neuries of radio active per dish the mutations are quite lethal.

Gamma rays

Active cobalt (Co^{16}) is one of the good sources. These radiations are electro-magnetic like x-rays but these are of shorter wavelength and hence more penetrating. These are also measured in terms of Roentgent unit.

The nature of induced mutation has been found that new genes or alleles induced by irradiations are of essentially the same kind as the spontaneous one. The only change here is increased in the rate of mutation caused by irradiation.

12.3.4. Non-ionizing radiations

Ultra violet rays with short wave length are absorbed by the materials causing non-ionizing radiations. These rays increase the energy levels of electron causing exitation which resulted molecules causes mutations. Though it is not deeply penetrating and applicable only to thin layers of cells. It has been found to be effective on pollen grains and root tips.

It has been found that non-ionizing radiations do not result generally into grass chromosomal rearrangement. On the contrary, x-rays radiation do so. Also ultra-violet radiation induced higher frequency of socalled point mutation per chromosomal aberration they do x-rays e.g., mutation found in Tradiscantia pollen and maize endosperm.

Chapter 13.

13. SOME EXAMPLES OF RESULT ACHIEVED WITH MUTATION BREEDING

Some examples of the results achieved in agriculture

It has often been pointed out that induced mutants may be of great value in cross-breeding programmes. Theoritical studies by Hogberg Mettin and others have shown that different members of a polygenic system governing an individual character can be changed by mutation.

Although the use of induced mutants in cross-breeding programmes and agriculture seems to offer great possibilities. The mutagens such as ionizing radiation as well as some chemical mutagens increase genetic variability, thus presenting the breeder with greater opportunities of controlling part of the evolution of his plants when subjecting them to breeding work.

Oka dealing with rice, shows that irradiation induced changes in the variability of polygenic traits. Scossiroli et al also demonstrate the irradiation increases the variability of most polygenic traits in wheat, in comparison with control.

Bhan and Kaul (1964) worked on frequency and spectrum of chlorophyll-deficient mutation in rice after treatment with radiation and alkylating agents.

Three varieties of rice were treated with gamma rays and two alkylating agents EMS and DES alone and in combinations with

a view to finding out the frequency and spectrum of chlorophyll mutations in relation to the genotype and the nature of the mutagens.

Among chlorophyll mutants in M_2 induced by radiations as well as alkylating agents, the albino type formed the majority class. EMS induced a significantly higher proportion of albinos than did gamma rays.

The highest mutation frequency for single treatments was obtained in Basmati treated with 40 KR gamma ray (4.21%) followed by 1.0% EMS treated Jhona (3.39%). Combined treatment of gamma rays and EMS to Jhona gave a chlorophyll mutation frequency as high as 5.48%. When the data for three varieties were pooled both alkylating agents and radiations induced a higher frequency of the albino type. The albino ratio for both classes was over 1.0 and combination treatment next frequent class closely followed by viridis for each of the three mutagens. Differences in the mutation spectrum produced by gamma rays and DES were negligible, EMS showing a higher albino ratio. The head progenies for 12796 plants which survived treatments with ionizing radiations yielded 176 independent reversions and 252 dependent reverions were obtained from 1976 plants which were tested following treatment with EMS. One should not attach much significance to these data because those revertants which arose in irradiated populations were obtained from a wide range of doses, whereas those obtained from EMS treatment were obtained from one selected concentration.

The first x-ray induced reversions were obtained in 1959, whereas the first EMS induced reversions were obtained in 1964. Accordingly, more genetic data are available on the 25 radiation induced reversions selected for concentrated study than on the 20 chemically induced reversions onwhich most effort is at present being expended.

T_2 data from crosses between the 25 radiation induced revertants, the yv mutant stock in which they originated and the normal green pro-genitor show that 22 of them are not available. Data from inter-crosses among the 25 revertants indicate that a minimum of nine different loci are responsible for reversion.

The data accumulated so far clearly show the following: A mutant (yv) which represses normal chlorophyll production in barley can be overcome by both dominant and recessive mutations. The recessive mutations have been induced with both ionizing radiations and EMS and those studied are all suppressors. The x-ray induced suppressors can be ascribed to a number of different chromosomes but several mutations have evolved in the same locus. The dominant revertants have all arisen from EMS treatment.

Singh, Dubey and Panda (1973) worked on mutation studies in T. aestivum (bread wheat) (2n = 42) is now considered an ideal test material for measuring rates of different types of mutations including point and deletion mutation and chromosomal rearrangements since it can withstand many of such changes which

have little or no detrimental effects on its viability.

Several species of wheat with different dwarfing genetic architecture were subjected to a series of mutagenic treatment including gamma rays, x-rays, thermal neutrons, radioisotopes and chemicals. These experiments have led to accumulation of information on (1) the relative efficiency of different treatments (2) genotype treatment interactions (3) effectiveness of single combined treatments (4) use of indicative parameter in M_1 (5) mutations at specific loci (6) dwarfing genes in wheat, (7) Mutation response to previous selection pressure and subsequent mutation trends and (8) effectiveness of mutation breeding in achieving specific objectives.

Raut, Panwar and Jain (1968) worked on mutation breeding in cotton. Photo in sensitive mutants: The variety MCU-5 recently released for cultivation in South India is one of our best quality cottons. Because of its photosensitive under North Indian conditions, it flowers very late and yields negligible quantity of seed cotton. A mutant of this variety isolated after gamma irradiation of seeds in photosensitive, flowers in 55-60 days yield as well or better than the standard variety of the locality and spins to 50s counts.

Aastveit (1965) studied effects of combinations of mutagens on mutation frequency in barley.

Two mutagens acting in sequence may produce higher or lower mutation frequencies than the sum of single treatments.

Since 1965 combination of mutagens have been tried in barley and Vicia faba. The present paper reports upon the result of three different barley experiments (a, b & c). The mutagen tested, single and in combinations of gamma rays, EMS, DES and neutrons. The combination of gamma rays and EMS gave less than additive frequencies of mutated M_1 plants for chlorophyll mutations. When estimated on a spike or 1000 M_2 plant basis, on the other hand, the same combination rates were estimated at mutants per 100 M_2 plants. The combined treatment gave the highest segregation ratio of Albino and Viridis mutants. These ratios are also increased significantly with increase doses of EMS. It is concluded that the over additivity of gamma rays and EMS may to some extent be a dose effect. All treatment with gamma rays, EMS, DES and neutrons, significant differences were also found in the mutation spectrum and in the segregation ratios. Neutron gave the highest relative frequencies of Albino mutants within segregating families. The mutation frequencies after combination other than gamma rays, EMS varied considerably.

Katarina Borojevic and S. Borojevic (1973) studied response of different genotypes of Triticum aestivum and sp. vulgare to mutagenic treatments. For the study of response of different genotypes to irradiation they had chosen several varieties of Triticum aestivum and sp. vulgare. The analysis through generations was performed on seven varieties representing entirel different genotypes and divided in three experiments. According

to the year and type of treatment the result shows that irradiation by thermal neutrons and x-rays and gamma rays result in a decrease of quantitative characters in the M_1 generation. The mean value increase sharply in M_2 compared with M_1 but are still below those of control. In M_3 a slight decrease was observed, in the M_4 a stagnation and in M_5 the mean values are around those of control in all genotype. If the individual genotypes are exceeded the mean of the control in the M_4 and M_5. It was very difficult to see the response of different genotypes. In general all genotypes react in the same way. The specific response to mutagenic treatments was not found in the studies of the quantitative character of the seven high selected varieties of Triticum aestivum vulgare in later generations.

Gustafsson, Ludquist and Khan (1973) studied yield analysis after repeated mutagenic treatment and selection in barley. Grains were treated for 1, 2 or 3 years, in 1960-62, either singly with neutrons (N) or ethyl methane sulphonate (E) or with combined treatment EN, NE, ENE, NEN simultaneously, a series with untreated and unselected (C) populations was used EEC, EC, CCC, NN, NC, NCC and all were compared with intensely selected control in 2 varieties. Chlorophyll mutations and their components of grain yield, grain per row, yield per plant, spike per row, spike per plant and plant per row and interaction of these components with location. N treatment give rise to selection definitely superior to E treatment combining N and E gave no advantage.

Sethi (1974) studied induced mutation of plant breeding significance in barley.

In order to obtain beneficial viable mutations in (164 barley (Hordeum sativum jess) dry dormant seeds were treated with different doses of EMS, gamma rays, 32 P and 32S. Twenty two of the 147 viable mutant types scored in the M_2 and confirmed in M_3 generation, viz., Dwarf, erectoides, stiff straw very early, early profuse tillering, synchronous tillering, late leaf - senescence, long broad leaf, dark green, thick stiff culm, male sterile, thick grain, double floret, brittle own, multinodosum cum-monopodial branching, many noded dwarf, thick culm dwarf, and long pendumcled dwarf, of plant breeding significance are described.

Minocha and Saini (1973) studied mutation frequency in barley following treatment with EMS or gamma rays and post treatment with GA.

Ethyl methanesulphonate and gamma radiation treated seeds of barley were soaked in distilled water or 1000 PPM gibberellic acid for two hours to study the effect of the post treatment on the chlorophyll mutation frequencies. Post soaking in water resulted in reduction in mutation frequencies. Mutation frequencies following mutagens plus GA treatments were slightly more as compared to mutagens plus water treatment. Maximum mutation frequencies, however, were obtained when no post treatment was given.

Das and Mukherjee (1973) studied effect of gamma radiation and ethyl methane sulphonate on seeds, cuttings and pollen in grapes.

Studies on the effect of acute gamma irradiation on seeds, cuttings and pollen of grapes have shown that an exposure of 2-6 Kr and 10 Kr. was optimum for unrooted, dormant cuttings and wet stratified seeds, respectively. No conclusive exposure data were obtained in regard to pollen irradiation. EMS treatment of seeds has indicated that a concentration of 0.2-0.5 would be effective. In a comparative study of seed treatment with gamma rays and EMS, EMS treatments were found to be less injurious to growth attributes as compared to gamma rays.

Narahari and Bora (1973) studied radiation induced spikelet abnormalities and mutations in rice. The occurrence of induced morphological changes in an irradiated population is a common phenomenon. The relative radiosensitivities of species in respect of these changes may perhaps be taken as an index of the degree of their genetic variability.

Extensive use of ionizing radiation has been made on the induction of cytogenetic effects in rice following the work of Ichijima (1934) and Ramiah and Parthasarthy (1938). During the course of studies on the differential radio sensitivities of several varieties of rice, morphological changes of a wide spectrum have been observed.

Saini and Sharma (1973) studied radiation induced variation in rice improvement. Dry seeds of two rice varieties viz., Jhona 349 and Punjab and Taichung-1 of Taiwan and F_2 of crosses between them were treated with 20 Kr, 30 Kr, 40 Kr and 50 Kr of gamma rays from a Co^{60} sources. To study the variability in R_2 generation, space plant rows from 40 randomly selected R_1 panicles of each treatment and the respective controls were grown in split plot design with three replications. The mean value of a character is 38 out of 48 treated populations remained unchanged inspite of increase in the variance while in the remaining 10 cases it recorded significant and unidirectional shift from that of the control. The genetic variance in the hybrid material was greater in magnitude than that obtained in the pure type for all the character and except for tillers per plant it further increased after irradiation, showing a supplementary effect to the variance normally released by hybridization. It was concluded that for effecting improvement in various economic characters of rice plant, hybridization followed by irradiation may be better than hybridization alone.

Goud (1966) studied induced mutation in bread wheat. To evaluate the efficiency of treatment with gamma rays, fast neutrous and EMS in producing mutations in qualitative characters in bread wheat as experiment was conducted with six varieties (N.P. 876, N.P. 872, N.P. 870, N.P. 863 and N.P. 862). Treated and control seeds of all the six varieties were sown in the field. The growth and survival was much hampered in EMS treatments 30 Kr. gamma irradiation. N.P. 870 was much less affected

by EMS treatment as compared to N.P. 876, while the later was relatively more resistant to gamma irradiation. About 10 and 35% of chimeras were observed in gamma and EMS treated plants respectively in M_1 generation. The spectrum and frequency of visible mutations were studied in M_2 generation. The chlorophyll and viable mutation frequency was very high in EMS treatments followed by gamma irradiations. The mutation frequency varied with variety N.P. 870 was highly mutable whereas N.P. 862 was rather stable with the other varieties falling in between gamma irradiation produced more of albino and speltoid mutations, whereas EMS produced more of Xantha, viridis subcompactoid, compactoid and sphaerococooid mutations. The mutation spectrum was considerably increased in EMS treatments.

Kaicker and Swarup (1972) studied induced mutation in roses. It has been possible to obtain mutations for flower colour in 3 cultivars of rose viz. Christion Dior, Queen Elizabeth and Kiss of Fire after gamma irradiation, which might be of direct commercial importance as they are found to deviate from other types. Five to ten Kr of gamma rays has proved to be the best for treatment of dormant buds.

Dormancy of buds induced after both gamma irradiation and chemical treatments 10 kr of gamma rays proved toxic to all the cultivars and treatments including NMU (N-nitroso n - methyl urethane) EMS (Ethyl methane sulphonate) E I (Ethylene amine)

in different concentrations in combination with (DMS sulphoxide) have been tried on growing plants. Dipping buds in NMU and EMS results in mutants for colour in Christian - Dior and reduction in petal number in kiss of Fire respectively. Improvement of rose, a very heterozygous plant with improvement of rose constitution through conventional breeding methods are difficult and slow and does not offer much success. Mutation in roses have been obtained through irradiation using x-rays by Gelin (1955) Broertsis (1965) and Chan (1966).

Lobana and Kewal Krishna Verma (1972) studied genetic regulation of chromosome pairing in Delphinium. The internuclear distribution of univalents was analysed in several partially asynaptic plants obtained from irradiated and inbred population of Delphinium. It was observed that in all the three inbred plants the distribution pattern deviated significantly from a normal or poisson type of distribution, one of these plants was found to show preponderence of two extreme classes of cells. One showing complete bivalent formation, the other showing all the chromosomes as univalents. This indicates that the control of chromosomes pairing and chiasma formation in a cell is based to some extent on qualitative rather than purely qualitative type of genetic determination. On the basis of all the observations it has been concluded that the genetic control of chromosome pairing and chiasma formation involves major genes and some modifiers.

Azatyan (1974) conducted cytogenetic analysis of the mutagen C - effect of trifunctional nitrogen mustard (HN^3) on dry Crepis capillaris L. seeds. Under the effect of trifunctional mustard (HN^3) on C. capillaris seeds produced a considerable number of chromatids aberrations. This is attributed to direct mutagen reaction with chromosomes in the period of initial growth (G1) causing potential changes which become true breaks at the time of their replication - M.D.S.

Ronnenkamp, Gorz and Haskins (1975) conducted genetic studies of induced mutants in Melilotus alba with reference to inheritance and complementation of six additional chlorophyll deficient mutant.

Sidoroya (1975) conducted study of natural and induced mutability of mutants (exemplified by P. sativum L. mutants).

To determine whether mutants differ from initial forms in character of hereditary variability, the rate and spectrum of natural and ethyl methane sulphonate induced mutations were studied in 4 mutants and the initial form of pea (Pisum sativum). The data showed that in peas, and probably in other culture. The selection of mutant forms automatically lead to an increase of the rate and broadening of the spectrum of hereditary variability and morphogenesis, is called destabilizing.

FUTURE PLAN OF WORK

Chapter 14.

FUTURE PLAN OF WORK

The future plan of work is

Induction of mutation
Induction of polyploidy
Cytological investigations and
Hybridization.

The taxa to be investigated are seven species of Acanthaceae as follows

Acanthus longifolius Linn.
Adhatoda vasica Nees.
Andrographis paniculata Nees.
Barleria cristata Linn.
Barleria lunulina Lindl.
Ruellia tiueediana Griseb.
Ruellia tuberosa Linn.
Thunbergia alata Bojer.

Induction of mutation

To study use and treatment of seed, seedlings and whole plant with physical and chemical mutagens, e.g. preparation of phosphate buffer stock solution, treatment with EMS, DES, Caffein etc. in phosphate buffer solution and other general methods.

Induction of polyploidy

Different methods of treatment of research material with colchicine for induction of polyploidy will be used.

Cytological investigations

For cytological investigations collection and fixation of material in suitable fixatives will be carried out. Acetocarmine stain will be prepared. Slides will be prepared with smear or squash technique. The study will include the different stages of cell division, e.g. prophase, metaphase, anaphase, telophase, linkage and crossing over, chiasma formation, chromosome morphology, crossing over, spontaneous and induced chromosomal aberrations, change in form and relative size of chromosomes, chromosomal association etc.

Hybridization

To study some major principles involved in a hybridization programme, the selection of the parental strains and a general method of handling the hybrid material etc. These are following aspects:

Selection of parental material
Handling of hybrid material
Early testing
Pedigree method
Back cross method
Multiple crosses
Combining ability
Interspecific and intergeneric crosses
Selfing
Emasculation
Pollination.

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